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CONTENTS

JANUARY, NO. 1

A spectrophotometric study of the changes in peroxide value of spray-dried whole milk powder during storage. HARRY PYENSON AND P. H. TRACY 1

Some physiological effects of extending the colostrum feeding period of dairy calves. T. S. SUTTON AND H. E. KAESER 13

Formic acid content of milk heated to high temperatures as determined by the distillation procedure. I. A. GOULD AND R. S. FRANTZ 27

Lactic acid in dairy products. IV. Effects of storage of evaporated milk on lactic and formic acid production, on pH, and on acidity and formol titrations. I. A. GOULD, EARL WEAVER, AND R. S. FRANTZ 33

Influence of homogenization of fat on haircoat of dairy calves. WILBUR BATE, DWIGHT ESPE, AND C. Y. CANNON 41

The effect of increasing the negative pressure and widening of the vacuum-release ratio on the rate of removal of milk from the udder. VEARL R. SMITH AND W. E. PETERSEN 45

The keeping quality of samples of commercially dried milk packed in air and in inert gas. GEORGE R. GREENBANK, PHILIP A. WRIGHT, EDGAR F. DEYSHER, AND GEORGE E. HOLM 55

Association announcement 63

Officers and committees of the American Dairy Science Association 64

Abstracts of literature A1

FEBRUARY, NO. 2

The occurrence and survival of Brucella abortus in cheddar and limburger cheese. H. L. GILMAN, A. C. DAHLBERG AND J. C. MARQUARDT 71

The calculation of total solids by means of the Sharp and Hart equation. B. L. HERRINGTON 87

The vitamin A and carotene content of market butter produced in Kansas. D. B. PARRISH, W. H. MARTIN, F. W. ATKESON, AND J. S. HUGHES 91

Some observations on the acid inversion of sucrose. THEO. R. FREEMAN 101

Studies of the carbohydrate metabolism of mammary gland tissue in vitro. II. The metabolism of citric acid and B-hydroxybutyric acid in tissue slices. C. B. KNODT AND W. E. PETERSEN 115

Studies of the carbohydrate metabolism of mammary gland tissue in vitro. III. Glycogen as an intermediary in the formation of lactose. C. B. KNODT AND W. E. PETERSEN 121

Association announcement 129

Abstracts of literature A25

MARCH, NO. 3

Studies on ketosis in dairy cattle. VII. The efficacy of B vitamins and methionine in the treatment of ketosis. J. C. SHAW 131

The estimation of the weight of bulls from heart girth measurements. CECIL BRANTON AND G. W. SALISBURY 141

The rate of autoxidation of milk fat in atmospheres of different oxygen concentraton. P. S. SCHAFFER, G. R. GREENBANK, AND G. E. HOLM 145

Studies on ketosis in dairy cattle. VIII. Spontaneous recovery. J. C. SHAW 151

Relation of corn and alfalfa silage to the quality of cheese and its carotene and vitamin A content. KIYOSHI HIGUCHI, W. V. PRICE, AND W. H. PETERSON 157
The vitamin content of mare's milk. ARTHUR D. HOLMES, BEULA V. MCKEY, ANNE W. WERTZ, HARRY G. LINDQUIST, AND LEONARD R. PARKINSON 163
Propionic acid, sodium propionate and calcium propionate as inhibitors of mold growth. II. Studies pertaining to the active agent responsible for the inhibitory effect of the propionates. J. C. OLSON, JR. AND H. MACY 173
Abstracts of literature A31

APRIL, NO. 4

Lactic acid, pyruvic acid, amino acids, acetone bodies, oxygen, carbon dioxide, and hemoglobin in arterial and mammary venous bloods of cows under various physiological conditions. J. C. SHAW 183
Effect of warm weather on grazing performance of milking cows. D. M. SEATH AND G. D. MILLER 199
The oxygen content of the atmosphere in containers of dried milk packed in nitrogen. P. S. SCHAFFER AND GEO. E. HOLM 207
Factors which contribute to the physical stability of frozen cream. R. W. BELL AND C. F. SANDERS 213
The effect of milk products on the heat-stability and viscosity of cream-style foods. B. H. WEBB AND C. F. HUFNAGEL 221
Controlled experiments on the value of supplementary vitamins for young dairy calves. C. L. NORTON, H. D. EATON, J. K. LOOSLI, AND A. A. SPIELMAN 231
The loss of nutrients in hay and meadow crop silage during storage. C. F. MONROE, J. H. HILTON, R. E. HODGSON, W. A. KING, AND W. E. KRAUSS 239
Announcement 257
Abstracts of literature A53

MAY, NO. 5

Live weight versus metabolic body size in dairy cows and goats. W. L. GAINES 259
The effect of route delivery on the flavor, riboflavin, and ascorbic acid content of milk. D. V. JOSEPHSON, L. H. BURGWALD, AND R. B. STOLTZ 273
The effect of sulfanilamide upon the livability and metabolism of bovine spermatozoa. C. B. KNODT AND G. W. SALISBURY 285
Vitamin C, hydrogen peroxide, copper and the tallowy flavor in milk. VLADIMIR N. KRUKOVSKY AND E. S. GUTHRIE 293
A simplified extraction-distillation method for the determination of the volatile fatty acids of cheese. K. L. SMILEY, F. V. KOSIKOWSKY, AND A. C. DAHLBERG 307
Defluorinated rock phosphate as a phosphorus supplement to the rations of dairy cattle. T. W. GULLICKSON AND FLOYD C. OLSON 317
Program—forty-first annual meeting 321
Abstracts of literature A67

JUNE, NO. 6

The influence of age on breeding efficiency of dairy cattle in artificial insemination. T. TANABE AND G. W. SALISBURY 337
Variation between breeds in feed cost of milk production. LYNN COPELAND 345
The effect of feeding cod-liver oil on the "oxidized" and "goaty" flavors and vitamin C in milk. E. S. GUTHRIE 349
The results of deaeration on the oxygen, vitamin C, and the oxidized flavors of milk. E. S. GUTHRIE 359

Relation of the temperature of separation and the heat treatment given the skim milk to the keeping quality of spray dried ice cream mix. HARRY PYENSON AND P. H. TRACY . 371
Carotene utilization in the newborn calf. A. A. SPIELMAN, J. K. LOOSLI, J. W. THOMAS, C. L. NORTON, AND K. L. TURK . 381
Abstracts of literature .. A73

JULY, NO. 7

Clipping vs. grazing by dairy heifers as means of estimating yield of bluegrass pastures. H. B. MORRISON AND FORDYCE ELY 393
A synthetic pabulum for the preservation of bull semen. PAUL H. PHILLIPS AND ROBERT R. SPITZER ... 407
Time required for making flavor judgments of milk. G. M. TROUT .. . 415
Heat coagulations of milk. WILFORD C. COLE AND N. P. TARASSUK 421
Mercurimetric method for salt determination in butter and new cheese. W. S. ARBUCKLE 431
Cryophilic organisms in water and butter. J. J. JEZESKI AND H. MACY . . 439
Measuring detergency functions as affected by various detergents and procedures against milk films by application of a mechanical washing apparatus. J. M. JENSEN . 453
The relative importance of high temperature and high humidity as factors influencing respiration rate, body temperature, and pulse rate of dairy cows. D. M. SEATH AND G. D. MILLER 465
Abstracts of literature A87

AUGUST, NO. 8

Abstracts of papers presented at the 41st annual meeting 473
Proceedings of the 41st annual meeting 559
Abstracts of literature A111

SEPTEMBER, NO. 9

Factors influencing acid production by cheese cultures: I. Effect of cooking temperatures on acid production in the manufacture of cheddar cheese. F. J. BABEL 589
Factors influencing acid production of cheese cultures: II. Influence of bacteriophage on acid production in the manufacture of cheddar and cottage cheese. F. J. BABEL 597
The manufacture of sterilized caramel milk. B. H. WEBB AND C. F. HUFNAGEL 607
Digestibility of kobe lespedeza hay. L. L. RUSOFF, D. M. SEATH, AND G. D. MILLER 613
Studies on milk fever in dairy cows: I. The possible rôle of vitamin D in milk fever. J. W. HIBBS, W. E. KRAUSS, C. F. MONROE, AND T. S. SUTTON 617
Additional observations on the stability of ascorbic acid and sodium L-ascorbate in evaporated milk. F. J. DOAN AND D. V. JOSEPHSON 625
Abstracts of literature . .. A137

OCTOBER, NO. 10

The use of urea in rations for dairy cows under Hawaiian conditions. E. L. WILLETT, L. A. HENKE, AND C. MARUYAMA 629
Moisture studies in dry products of milk. I. Kinetics of the dehydration of alpha lactose hydrate in toluene. R. P. CHOI, C. M. O'MALLEY, AND B. W. FAIRBANKS 639

Colorimetric determination of ammonia in milk and dry products of milk. R. P. Choi, C. M. O'Malley, and B. W. Fairbanks 645
The relationship of the growth of all bacteria and coliform bacteria in pasteurized milk held at refrigeration temperatures. A. C. Dahlberg 651
The effect of pregnancy on the body weight of dairy cows. Dexter N. Putnam and H. O. Henderson 657
Studies on the chemical composition of calf blood. A. E. Teeri, H. A. Keener, and K. S. Morrow 663
The effect of vitamin A deficiency on reproduction in dairy bulls. R. E. Hodgson, S. R. Hall, W. J. Sweetman, H. G. Wiseman, and H. T. Converse 669
Methods of testing 80 per cent cream for butterfat. Burdet Heinemann 689
Fertility of bull semen diluted at 1:100. G. W. Salisbury 695
Frozen homogenized milk. 1. Effect of freezing and storage temperature on the physical characteristics of homogenized milk. Lieutenant Colonel C. J. Babcock, SNC, AUS, Major Richard N. Roerig, SNC, AUS, Captain Joseph N. Stabile, SNC, AUS, Captain William A. Dunlap, SNC, AUS, and Colonel Raymond Randall, VC, USA 699
The placental transmission and fetal storage of vitamin A and carotene in the bovine. A. A. Spielman, J. W. Thomas, J. K. Loosli, C. L. Norton, and K. L. Turk 707
Differential nitrogen retention from casein, lactalbumin, and soy protein, and hydrolysates therefrom. G. C. Supplee and Esther L. Clark 717
Studies of the effect of heat on milk dialysates. C. L. Hankinson, M. E. Ellertson, and Wm. Gleim 727
Abstracts of literature A161

NOVEMBER, NO. 11

Modification of the phosphatase test as applied to cheddar cheese and application of the test to fluid milk. George P. Sanders and Oscar S. Sager 737
Bactericidal properties of some surface-active agents. W. S. Mueller, Emmett Bennett, and James E. Fuller 751
The effect of vitamin supplements upon survival of new-born calves. R. Gaurth Hansen, Paul H. Phillips, and I. W. Rupel 761
Studies on milk fever in dairy cows. II. The effect of vitamin D on some of the blood changes in normal and milk fever cows at parturition. J. W. Hibbs, W. E. Krauss, W. D. Pounden, C. F. Monroe, and T. S. Sutton 767
The pantethenic acid, niacin and biotin content of commercial and experimental milks. J. J. Stefaniak and W. H. Peterson 783
The carotene and vitamin A contents of Wisconsin cheese. Kiyoshi Higuchi and W. H. Peterson 789
Some observations on "quality" in hays. J. G. Archibald, E. Bennett, and J. W. Kuzmeski 795
Abstracts of literature A169

DECEMBER, NO. 12

Shark meal as a protein supplement in dairy calf rations. Sidney P. Marshall, R. B. Becker, and Geo. K. Davis 801
Colostrum milk and its vitamin A content. R. G. Hansen, P. H. Phillips, and V. R. Smith 809
The effect of certain coal-tar dyes used for semen identification on the livability and fertility of bull spermatozoa. J. O. Almquist 815
Influence of nonfat dry milk solids on the nutritive value of bread. L. K. Riggs,

ANNABEL BEATY, AND ARNOLD H. JOHNSON 821

Blood studies of Louisiana dairy cows. II. Calcium, inorganic phosphorous, hemoglobin value, erythrocyte count, leucocyte count and differential leucocyte percentages. L. L. RUSOFF AND P. L. PIERCY . . 831

The effect of cottonseed in the ration on percentage of fat and serum solids content of milk. R. N. DAVIS AND F. G. HARLAND . 839

Babcock vs. Mojonnier test for homogenized and unhomogenized milk. F. G. HARLAND AND R. N. DAVIS 845

Storage and treatment of milking machine inflations. J. M. JENSEN AND A. L. BORTREE . . 849

A rapid direct-distillation method for determining the volatile fatty acids of cheese. F. V. KOSIKOWSKY AND A. C. DAHLBERG . 861

Circulation of the Journal of Dairy Science . 873

Author Index of Original Articles . 915

Subject Index of Original Articles .. 923

Abstracts of Literature . . A191

Author Index of Abstracts A209

Subject Index of Abstracts . A219

Editorial Board ii

Table of Contents . iii

Errata . . viii

ERRATA

Vol. XXIX, No. 4, page A55. Abstract No. 106. The fifth sentence of this abstract should read: Cheese from milk pasteurized at 143° F. for 30 minutes or 162° F. for 15 seconds did not give values greater than 5 units, regardless of the age of the cheese.

Abstract #170, Vol. XXIX, No. 6, June, 1946, page A84—title should read: Bulk Freezing for Commercial Use.

JOURNAL OF DAIRY SCIENCE

VOLUME XXIX JANUARY, 1946 NUMBER 1

A SPECTROPHOTOMETRIC STUDY OF THE CHANGES IN PEROXIDE VALUE OF SPRAY-DRIED WHOLE MILK POWDER DURING STORAGE

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Of the fatty acids present in milk (1), oleic and linoleic acids, being unsaturated, are probably most concerned in the oxidative changes that result in the production of oxidized flavor. It is thought by some that phospholipid material usually associated with the butterfat may also play an important rôle in oxidative changes.

Butterfat displays a certain initial resistance to oxidation. It then passes through a period during which there is little or no absorption of oxygen. This period is termed "the period of induction" and when it is over, the fat will absorb oxygen at an increasingly rapid rate. During the induction period combination with oxygen is slow and the resulting deterioration in flavor is usually slight. The phenomenon of the induction period is considered to be due to the action of traces of protective substances known as antioxidants or inhibitors, which delay oxidation and prevent the reaction between oxygen and the fat from attaining its full velocity. Natural fats contain traces of non-fatty compounds. These substances lengthen the induction period but in time lose their protective action and are themselves oxidized to a non-antioxygenic form.

A period of active oxygen absorption follows the period of induction. Linoleic acid has an extremely rapid rate of oxygen absorption but there is only 3–4 per cent of it present in butterfat. The oleic acid oxygen absorption rate is decidedly slower than the linoleic acid, but butterfat contains 25–40 per cent of oleic acid.

The process of fat deterioration resulting from oxidation is not well understood, but it is thought that several reactions occur simultaneously. The peroxides formed are an intermediate product of fat oxidation and are by no means the end product of the reaction. Further products of fat oxidation are thought to be aldehydes, ketones, keto hydroxy compounds and epihydrin aldehyde. Glycerol disappears practically as fast as it is formed, being oxidized immediately on formation.

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Peroxide values have been used with some success in determining the extent of oxidation of fats and oils in a pure or nearly pure state (5, 11). There are numerous methods that have been proposed for the detection and estimation of the degree of oxidation. Most of these methods, based on iodometric titrations, work out fairly well where pure or nearly pure fats and oils are used. In the examination of a material such as milk powder, in which the fat is intimately associated with other milk constituents, it is desirable to extract the fat by procedures least likely to decompose the fat-peroxides as they are intermediate products of oxidation.

Organoleptic methods are commonly used to detect off flavors in whole milk powder. In many pure fats and oils the keeping quality is determined by the length of induction period under controlled conditions. The longer the induction period the better the keeping quality of the fat or oil in question. Therefore, it was thought desirable to study the relationship beween peroxide value and the palatability or keeping quality of spray-dried whole milk powder.

REVIEW OF LITERATURE

Dahle and Nelson (4) studied antioxidants in dry milk fat. Their influence was measured by means of the peroxide number determined by the procedure of Greenbank and Holm (5). They found that the induction period may vary as much as four days between identical tubes of fat under the conditions employed.

Hollender and Tracy (10) used Smith's (14) method for the determination of peroxide values on roll-dried whole milk powder. They concluded from their study that: 1) the peroxide value cannot be used to detect early oxidation of the fat; 2) it may be used to show oxidation of a high degree; 3) the development of oxidized flavor in milk powder can be detected at an earlier stage by the sense of taste than by the use of the peroxide test; 4) the peroxide test could not be used to predict the keeping quality of a fresh sample of powdered whole milk.

Lea, Moran and Smith (11) using roll and spray whole milk powder showed that with the disappearance of free oxygen from the container both the peroxide and Kreis values passed through a maximum and fell away again to low values.

Greenbank, Wright, and Deysher (6) made a study of the effect of inert gas on the keeping quality of commercially manufactured and packed dried whole milk. They used flavor and peroxide values as indices of the keeping quality and conclude that in the air-packed samples there was good correlation between peroxide value and flavor, while in the nitrogen-packed samples the correlation was less evident.

Chapman and McFarlane (2) claim that there is a correlation between fat peroxide content and keeping quality in powdered whole milk when using the colorimetric method which is based on the oxidation of ferrous to ferric iron.

Pyenson, Tracy and Trimble (13) analyzed 180 samples of spray-dried whole milk powder varying in age from fresh to 21 months for peroxide content and gas content. The reconstituted milk was also scored for flavor. They concluded that the peroxide value is not a satisfactory criterion of the palatability or keeping quality of spray-dried whole milk powder.

Holm (9) recently stated that, "the rate of oxidation of fats may be measured by that rate of formation of peroxides only if the oxidation takes place at relatively low temperatures and if the peroxide values are relatively small.

"At higher temperatures the rate of peroxide formation may not be a measure of the rate of oxygen absorption and the extent of peroxide formation may not necessarily be a measure of deterioration. This is especially true in the case of fats and oils which contain acids of high degree of unsaturation.

"The most satisfactory and practical method of measuring deterioration is by the time of appearance of off-odors and off-flavors, which are caused by the breakdown of peroxides."

EXPERIMENTAL PROCEDURE

Four commercial samples of spray-dried whole milk powder were obtained for this experiment. The batch numbers, dates of manufacture and age of powder when packed for gassing are as follows:

Batch No.	*Date of manufacture*	*Age of powder when gassed (days)*
A27	Jan. 27, 1944	15
A28	Jan. 28, 1944	14
A31	Jan. 31, 1944	11
B 2	Feb. 2, 1944	9

The powders were kept in air-sealed cans until they were gassed on February 11, 1944. Approximately 100 grams of powder was placed in each can. Part of the cans of each batch were nitrogen-packed and one-half of the samples were stored at 35° F. and the remainder at 90° F. for the first four months and then at 100° F. for the remainder of the storage period.

The processing data for the four batches of powder are as follows:

Batch no.	A27	A28	A31	B2
Composition of Condensed Milk				
Fat, per cent	10.72	10.53	10.33	10.93
Total solids, per cent	40.16	39.14	38.71	41.32

	Drying Data			
Inlet temperature	345° F.	345° F.	345° F.	345° F.
Outlet temperature	185° F.	185° F.	185° F.	185° F.
Nozzle number	58	58	64	58
	Powder Data			
Flavor	Good	Good	Good	Good
Color	Light	Light	Light	Light
Fat, per cent	26.04	25.98	26.25	26.17
Total solids, per cent	97.82	98.00	97.80	97.80
Moisture, per cent	2.18	2.00	2.20	2.20
Copper, p.p.m.	1.23	1.30	1.23	1.20
Iron, p.p.m.	6.3	5.3	4.2	4.7

The colorimetric method of Chapman and McFarlane (2) was used for the determination of fat peroxides using a Coleman Universal Number 11 Spectrophotometer. The authors claim it is more sensitive than iodometric methods.

For the determination of gas content of the headspace gas the method of Van Slyke and Sendroy (15) was used.

All the samples were scored for flavor at various intervals by at least two judges. The samples were scored for flavor within four hours after reconstitution.

Copper was determined by the method of Hetrick and Tracy (8) and iron was determined by the method of Pyenson and Tracy (12).

EXPERIMENTAL RESULTS

Changes in carbon dioxide concentration in headspace gas. Figure 1 shows the changes of carbon dioxide content of the headspace gas in the four samples over a period of one year. The production of carbon dioxide throughout the storage period was not uniform. Carbon dioxide is probably produced as the storage period of the powder progresses but is likely reabsorbed in some cases resulting in variable results.

The samples stored at 35° F. produced less carbon dioxide than those stored at 100° F. The greatest amount of carbon dioxide was produced in the air-packed samples stored at 100° F. The least carbon dioxide was produced in the nitrogen-packed samples stored at 35° F. The headspace gases varied in carbon dioxide from none to 0.61 per cent.

Changes in oxygen concentration in headspace gas. Figure 2 gives the data for the change in the percentage of oxygen in the headspace gas of both the air-packed and nitrogen-packed samples. Sixteen hours after packing and gassing the samples had the following analysis for oxygen:

Sample No.	*Per cent oxygen in headspace*	
	Air pack	*Nitrogen pack*
A27	20.84	2.43
A28	20.83	1.61
A31	20.81	2.05
B 2	21.05	1.88

It is a well-known fact that powdered milk on storage will show a reduction of oxygen in the headspace, the amount of change depending on a number of factors in the manufacture, packing and storage of the powder. The four commercial batches of whole milk powder used in these experiments were not packed directly from the drier but varied in age from 9 to 14 days at the time of packaging. From the results obtained in comparison with other experimental data the reduction in percentage of oxygen is not as great in the headspace gas of powder packaged cold as in powder packaged directly from the drier (3, 7).

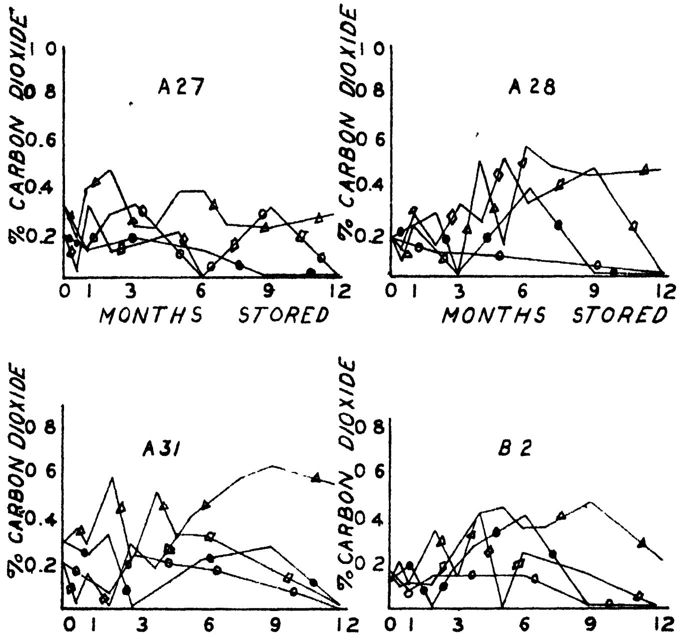

FIG. 1. Effect of storage on the carbon dioxide content of the headspace of spray dried whole milk powder. Legend to curves: ● air-packed stored at 35° F. ○ nitrogen-packed stored at 35° F. △ air-packed stored at 100° F. □ nitrogen-packed stored at 100° F.

The air-packed samples stored at 35° F. showed a good deal less oxygen absorption than the air-packed samples stored at 100° F. The maximum absorption at 35° F. was 3.59 per cent with an average absorption of 2.85 per cent oxygen. At the higher storage temperature there was a maximum drop of 9.98 per cent oxygen in the headspace with an average drop of 7.65 per cent oxygen for the four samples. There was approximately 2.7 times as much oxygen absorbed at 100° F. as at 35° F. after 12 months storage.

In the nitrogen-packed samples there was a similar trend in absorption between low and high temperature of storage. At 35° F. the nitrogen-packed samples averaged only 0.37 per cent oxygen reduction while at 100° F. there was an average reduction of 1.24 per cent of oxygen in 12 months. There was approximately 3.3 times as much oxygen absorbed at 100° F. than at 35° F. in the nitrogen-packed samples.

Changes in peroxide values. Peroxide values (expressed as milliequivalents of peroxide per kilogram of milk powder) were determined on the four samples of whole milk powder at frequent intervals throughout the storage period. It will be noted in figure 3 that all the samples had a peroxide value at the first determination except B2 stored at 35° F. and 100° F.

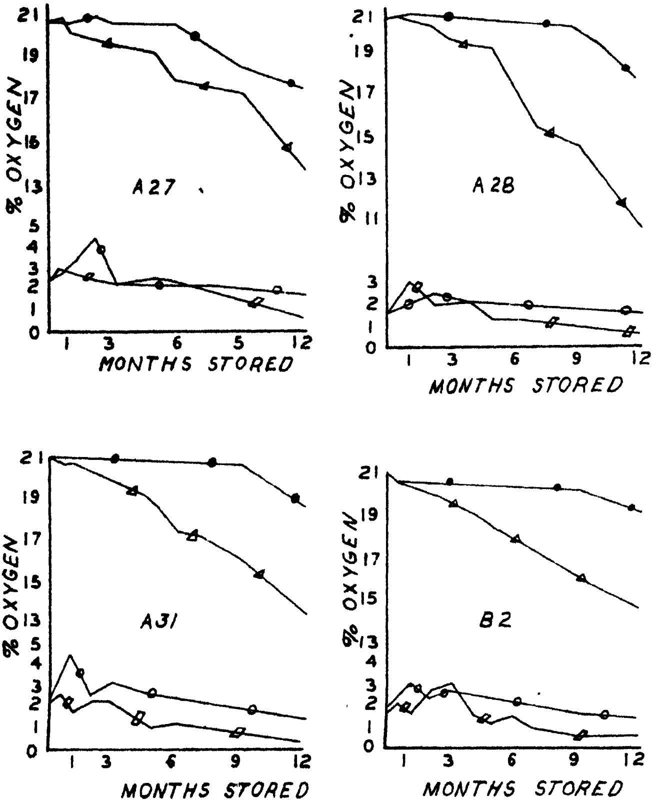

FIG. 2. Effect of storage on the oxygen content of the headspace of spray dried whole milk powder. Legend to curves: Same as in Fig. 1.

For the first three months of storage the peroxide values remained fairly low, fluctuating from 0 to 10 milliequivalents of peroxide per kilogram of milk powder. At four months storage, in most cases, the peroxide content increased sharply and reached a maximum at six to nine months. After nine months of storage the peroxide values started to decline and at twelve months most of the samples reached low values. On the whole these values were not as low as those recorded for the first three months of storage.

There seemed to be no significant differences in peroxide values between cold or hot storage or between air- and nitrogen-packed samples of the same powder at the same storage period. At 100° F. there was a tendency for the peroxide values of the whole milk powder to remain at the maximal or near the maximal values for a longer period of time than those peroxides produced at 35° F. It is noted from figure 3 that there was a gradual increase in maximal peroxide values as the age of the powder increased from 9 to 15 days before gas-packing. The highest peroxide value was obtained on sample A27 which was air-packed and held at 100° F. for six months.

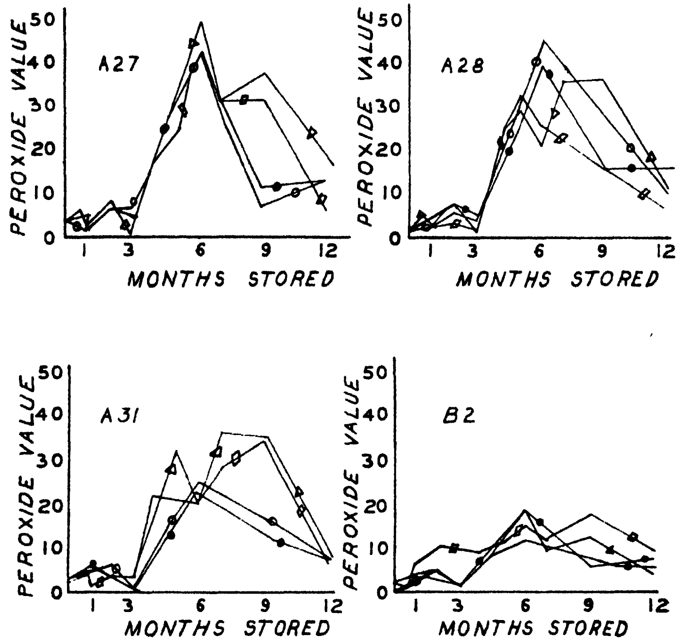

Fig. 3. Effect of storage on the peroxide value (milliequivalents of peroxide per kilogram of milk powder) of spray-dried whole milk powder. Legend to curves: Same as in Fig. 1.

Changes in flavor on storage. Whole milk powder that becomes oxidized seems to go through a certain number of preliminary changes in flavor on storage. A freshly prepared sample of whole milk powder will usually have a cooked flavor. As the period of storage increases a stale flavor usually develops. Sometimes a bitter off flavor may develop, followed by a metallic flavor, particularly in the case of powder stored at a low temperature. The four samples of powder studied here under various conditions of packing and storing went through a series of flavor changes. Not all of the samples developed every one of the off flavors mentioned but they all acquired an oxidized flavor before the end of the study.

Figure 4 shows that the powder stored under nitrogen at 35° F. kept the best, followed by air-packed at 35° F., nitrogen-packed at 100° F. and then air-packed at 100° F. Air-packed samples stored at 35° F. kept better than nitrogen-packed samples stored at 100° F. In every case nitrogen-packed samples kept better than the equivalent air-packed samples.

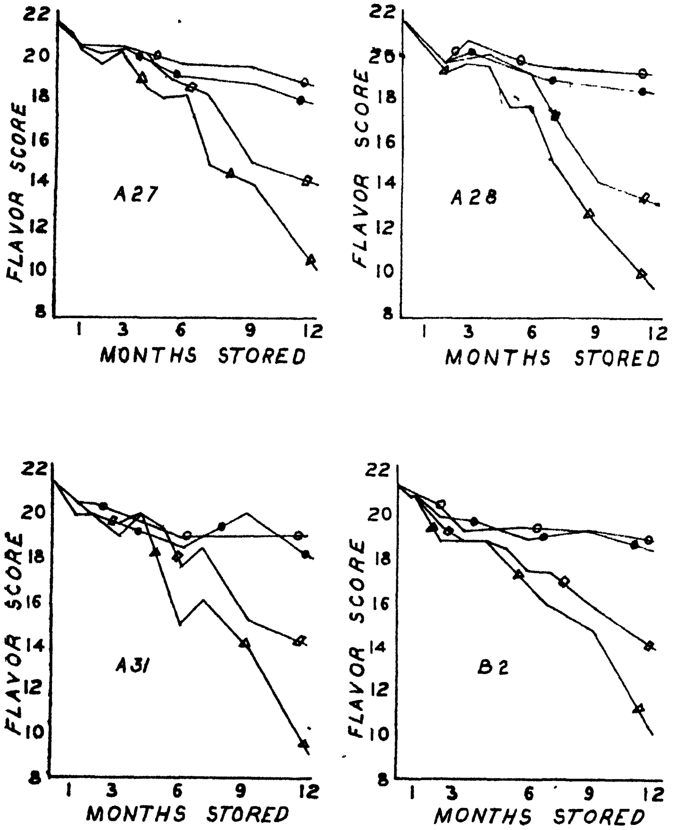

FIG. 4. Effect of storage on the flavor score of spray-dried whole milk powder. Legend to curves: Same as in Fig. 1.

Relation of peroxide value and flavor. A comparison of figures 3 and 4 show that there is no correlation between peroxide value and flavor score in either air- or nitrogen-packed samples stored at either 35° F. or 100° F. After the oxidized flavor developed the defect became progressively worse as the storage period lengthened but the peroxide values did not increase accordingly. The relation between oxidized-flavor development and peroxide value is summarized in table 1.

It will be noted from table 1 that some of the samples did not have an oxidized flavor when the greatest amounts of peroxide were present. The oxidized flavor did not develop in some of the samples until the peroxide value had fallen to a low figure after reaching its maximum. This would indicate that the oxidized flavor may be due to decomposition products formed after the peroxides occur and is not due to peroxide accumulation.

TABLE 1

Relation between oxidized flavor development and peroxide value

Sample*	Month oxidized flavor first noted	Peroxide value when oxidized flavor first noted	Month maximum value reached	Maximum peroxide value obtained	Flavor score at 1 yr. old	Peroxide value at 1 yr. old
A27035	12	13.1	6	42.8	17.5	13.1
A27135	12	13.4	6	42.8	18.5	13.4
A270100	4	17.7	6	48.8	10.0	16.6
A271100	5	23.9	6	42.8	14.0	5.7
A28035	12	17.2	6	39.7	18.0	17.2
A28135	12	10.3	6	46.4	19.0	10.3
A280100	5	28.8	9	36.3	9.0	11.7
A281100	6	24.9	9	42.0	13.0	7.5
A31035	6	23.5	6	23.5	18.0	8.3
A31135	6	25.2	6	25.2	19.0	7.0
A310100	5	32.8	9	36.7	9.0	7.9
A311100	6	20.2	9	34.5	14.0	6.6
B2035	6	18.9	6	18.9	18.5	7.6
B2135	6	12.6	6	12.6	19.0	6.9
B20100	6	15.4	9	18.6	10.0	9.6
B21100	6	18.8	6	18.8	14.0	4.4

* Key to samples: Fist letter and two numerals are sample numbers. Next numeral stands for air-packed (0) or nitrogen-packed (1). The last two or three numerals are storage temperature. 35 for 35° F. and 100 for 100° F.

DISCUSSION

Although the spectrophotometric method may be more sensitive than other methods for the determination of fat peroxides in whole milk powder, and gives higher results than iodometric methods (2) there still are some limitations in the measurement of peroxides by this procedure.

In the course of this study a number of factors arose that may or may not be important in determining peroxides in dry whole milk by this method. They may be listed as follows:

1. The dry whole milk is extracted by direct treatment with acetone. This procedure may not extract all of the butterfat. Lea, Moran and Smith (11) state, "The amount of fat which can be extracted from spray-dried full-cream milk powder by direct treatment with solvents is only a small proportion (ca. 3–14%) of that present, although in the case of the roller-dried powder extraction is almost complete (92–96%)."

2. Refluxing of the sample for 20 minutes at 65–70° C. may decompose

the fat peroxides and the unstable peroxides may decompose before extraction is accomplished and the determination made.

3. The size of the sample used in the method is only 200 milligrams and may not be representative.

4. Small changes in concentration of the water during the preparation of the sample may give false results.

5. Acetone precipitates some of the phospholipid material during the extraction. This phospholipid material left behind may be just as important in peroxide and oxidation development as the extracted fat.

From this study it is apparent that the major problem encountered in any method for the determination of peroxides in dry whole milk is the ability to extract the butterfat from the powder without changing it chemically. In work with pure butterfat this problem is not important as the butterfat has been extracted from the material before the peroxides have begun to develop. In dry whole milk powder where the fat is intimately associated with the milk solids-not-fat the problem of removing the fat without any chemical changes taking place is a difficult one. The phospholipid material associated with the fat should also be extracted for the determination as there is no definite proof that this substance is not associated with oxidative changes on storage.

Mechanical methods of extraction of the butterfat and phospholipid material have not been tried, but further investigations may show that some such procedure may be preferable for preparing the sample for analysis.

SUMMARY

One-hundred-gram samples of four different lots of powdered whole milk were stored at 35° and 100° F. for a period of one year. Half the samples were air-packed and the remainder were nitrogen-packed. Determinations were made periodically of the oxygen and carbon dioxide present in the headspace gas. Peroxide values were also measured and the reconstituted milk was judged for flavor. Summarized results follow:

1. The amount of carbon dioxide evolved during storage was not uniform, suggesting that this gas is reabsorbed to some extent by certain constituents of the milk. Higher carbon dioxide values were obtained on the samples stored at 100° F.

2. After twelve months storage there was approximately 2.7 times as much oxygen absorbed at 100° F. as at 35° F. in the air-packed samples. In the nitrogen-packed samples there was approximately 3.3 times as much oxygen absorbed at the higher storage temperature.

3. Most of the freshly packed samples showed some peroxide value though a number of these samples did not develop an oxidized flavor until after nine months of storage.

4. Although fluctuating peroxide values were obtained on the same lot

of powder during storage, in general, maximum values were reached in 6 to 9 months, after which the trend was downward.

5. There was no correlation between peroxide value and either the temperature of storage or the method of packing the powder.

6. The flavor score of all samples decreased during storage but at a more rapid rate in the case of the air-packed samples and those samples held at 100° F.

7. Although peroxide formation in all samples was higher after 12 months of storage, the values were not sufficiently correlated with flavor scores to justify the use of the peroxide test as a means of measuring or predicting the keeping quality of whole milk powder.

8. Organoleptic tests remain the most accurate method of measuring changes that occur in flavor and odor of powdered whole milk during storage at either 35° F. or 100° F.

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SOME PHYSIOLOGICAL EFFECTS OF EXTENDING THE COLOSTRUM FEEDING PERIOD OF DAIRY CALVES

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Within recent years considerable attention has been given to the value of colostrum as a feed for newborn animals. The Associates of Rogers (2) have reviewed the work relating to the immunizing properties of colostrum in protecting the young animal against various infective organisms common to the environment of the newborn calf. There are, however, in addition to these immunizing agents, other factors of similar importance in the nourishment of the newborn. Vitamin A is one of these factors that has received some investigational attention.

In 1933 Dann (4) reported that colostrum was many times as potent in vitamin A as normal milk. This finding has been adequately substantiated by other workers (6, 12, 13, 14, 15). The importance of vitamin A in the nutrition of the newborn calf has been shown (8, 11) and the value of colostrum in supplying this vitamin to the calf investigated. The newborn calf has a low vitamin A reserve—as measured by the carotene and vitamin A content of the liver (7) and blood plasma (8, 10). Colostrum is the natural source of vitamin A and carotene for the newborn animal. In the normal calf the amount of vitamin A and carotene in the blood rapidly increases following the ingestion of colostrum. In some instances, calves have apparently failed to respond to colostrum feeding (10). In others, either because of an insufficient quantity of vitamin A in the dam's colostrum, a failure to consume sufficient colostrum, or a failure to respond to colostrum feeding, benefit has been derived from direct vitamin A supplementation (8, 11).

The average dairy cow usually produces much more colostrum than her calf can consume. This colostrum, even that from cows on winter feed, is an important and perfectly adapted source of vitamin A, as well as of other essential factors for the young calf. Yet, on most dairy farms the colostrum not nursed by the calf is wasted or used for feeding purposes in which the greatest benefits from the vitamin A and other factors are not obtained. Allen (1) used stored colostrum to replace marketable milk in the feeding of calves. He found that colostrum could be stored in a frozen condition for as long as a year and still be entirely satisfactory as a substitute for marketable milk in calf raising.

The investigation presented in this report was undertaken to determine the effects of extending the colostrum feeding period to 7 days on the blood-

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plasma carotene, vitamin A, and ascorbic acid levels and on the health and general performance of dairy calves.

EXPERIMENTAL

Experimental animals. Thirty-six dairy calves born in The Ohio State University Dairy Herd during the winter feeding period of 1944–1945 were used in this experiment. Four dairy cattle breeds were represented as follows: 8 Ayrshire, 8 Guernsey, 8 Jersey and 12 Holstein-Friesian. Since all of the calves in The Ohio State University Dairy Herd were being used on a large-scale experimental test to determine the value of a commercial vitamin capsule in the prevention of calf scours, four groups were planned as follows:

Group I. Calves in this group were to receive colostrum for the usual period (3 days) plus a placebo capsule for the first 21 days.

Group II. These calves were to receive colostrum for 7 days plus a placebo capsule for 21 days.

Group III. Calves in this group were to receive colostrum for 3 days plus a vitamin capsule for 21 days.

Group IV. These calves were to receive colostrum for 7 days plus a vitamin capsule for 21 days.

The vitamin capsule used in this experiment contained 10,000 International Units of vitamin A, 300 U.S.P. Units of vitamin D, 50 milligrams niacin and 250 milligrams of ascorbic acid.

As calves were born they were assigned to a group following a definite order so that there was equal breed representation in each group. Thus, each group finally consisted of 2 each of the Ayrshire, Guernsey and Jersey breeds and 3 of the Holstein-Friesian breed. The original plans were to leave the calf with the dam for the first 3 days and to obtain the colostrum not nursed by the calf by milking for storage and later feeding. After 3 days the calf was to be weaned and fed whole milk or stored colostrum as the case might be, depending on the group to which the calf was originally assigned at birth. After 2 calves had been assigned to each group this plan was modified because a truly representative composite sample of colostrum for vitamin A and carotene determination could not be obtained from a cow being nursed by a calf and the colostrum intake of the calf could not be accurately determined.

The remaining 28 calves in the experiment were therefore taken from the dam immediately after they were dropped and fed from a nipple pail. They received colostrum from their own dam for the first 3 days, after which they received mixed Holstein milk or stored colostrum, depending upon the group to which they were assigned. The stored colostrum was usually a mixture from several cows, therefore in calculating the vitamin A and carotene intake of the colostrum-fed calves from the fourth to the seventh day the average potency of the colostrum from the entire group of cows was used. The potency values of the colostrum from cows which were nursed (8 cows) and those which had milk fever (3 cows) wère not used in determining this average potency because of the inability to obtain reliable representative samples as previously pointed out. Data on the potency of the colostrum fed to the calves in this experiment are reported in a previous paper (15). The potency values for average mixed Holstein milk produced during the winter months were used in determining the vitamin A and carotene intake of the milk-fed calves from the fourth to the seventh day. These potency values for the colostrum fed from the fourth to seventh day, inclusive, were: vitamin A, 821 micrograms; and carotene, 893 micrograms per pound; for the milk, the potency values were 126 micrograms of vitamin A and 143 micrograms of carotene per pound. The colostrum or milk was fed at the rate of one pound for each 10 pounds of body weight.

Techniques. All the calves were bled by puncturing the jugular vein before they nursed or were fed colostrum (2 exceptions) and on the 3rd, 5th, 7th, 9th, 11th, and 14th day of age, and 24 of the calves were bled when 21 days of age. A 30-ml. sample was drawn into a brown glass bottle containing 0.5 ml. of a 20 per cent potassium oxalate solution. The blood samples were taken to the laboratory immediately and in the majority of cases the determinations were completed within 4 hours.

Blood-plasma vitamin A and carotene were determined by the method of Kimble (5); ascorbic acid was determined by the macro method of Mindlin and Butler (9). The vitamin A and carotene in the colostrum fed were determined by the method of Boyer, *et al.* (3).

An Evelyn Photoelectric Colorimeter with the appropriate filters for each determination was used. The instrument previously had been standardized against crystalline vitamin A alcohol in chloroform solution, crystalline beta carotene in petroleum-ether solution, and ascorbic acid crystals in 3 per cent meta-phosphoric acid solution.

The calves were fed colostrum or milk at the rate of one pound daily for each 10 pounds of body weight and were weighed daily for the first 14 days. After reaching approximately 2 weeks of age, hay was available to the calves at all times.

EXPERIMENTAL RESULTS

Vitamin A intake. Data on the vitamin A intake of the calves used in this experiment are presented in tables 1 and 2. Table 1 shows the vitamin A and carotene intake from the colostrum alone in terms of micrograms. These intakes are reported as totals for the first 3 days and the next 4 days, because during the first 3 days all calves received colostrum from their dams and during the next 4 days those in groups II and IV received mixed stored colostrum and groups I and III received mixed milk.

TABLE 1

The vitamin A and carotene intake (from colostrum) of the calves during the first 7 days of the experiment

Quantities expressed in micrograms

Calf No.‡	1st 3 days		4th–7th days incl.		Total for first 7 days	
	Vitamin A*	Carotene	Vitamin A*	Carotene	Vitamin A*	Carotene
Group I: Control, colostrum 3 days—placebo capsule 21 days						
310A	13,512	14,708	4,032	4,576	17,544	19,284
314A	13,107	14,362	3,528	4,004	16,635	18,366
291G†	11,755	26,341	3,528	4,004	15,283	30,345
298G	8,790	21,000	3,024	3,432	11,832	24,432
449H†	17,843	15,489	4,032	4,576	21,875	20,065
459H	15,301	14,013	4,356	5,148	19,657	19,161
463H	17,050	15,320	5,020	5,720	22,070	21,040
330J	18,273	14,035	3,528	4,004	21,801	18,039
335J	9,042	9,672	3,024	3,432	12,066	13,104
Average	13,854	16,104	3,786	4,322	17,640	20,426
Group II: Experimental, colostrum 7 days—placebo capsule 21 days						
309A	36,640	29,612	32,840	35,720	69,480	65,332
313A	22,992	20,456	26,272	28,596	49,264	49,052
290G†	11,755	26,341	26,272	28,596	38,027	54,937
296G	12,750	31,650	19,704	21,432	32,454	53,082
450H†	17,843	15,489	25,451	27,683	45,294	43,172
457H	15,730	16,160	32,840	35,720	48,570	51,880
465H	24,300	22,102	32,840	35,720	57,140	57,822
329J	18,736	33,904	26,272	28,596	45,008	62,500
334J	21,405	16,801	22,988	25,004	44,393	41,805
Average	20,239	23,613	27,287	29,674	47,528	53,287
Group III: Experimental, colostrum 3 days—vitamin capsule 21 days						
308A	14,536	10,608	4,032	4,576	18,568	15,184
312A	9,208	5,712	3,528	4,004	12,736	9,716
293G	11,008	28,008	3,654	4,147	14,662	32,155
295G	15,960	26,092	2,016	2,288	17,976	28,380
448H†	17,843	15,489	4,032	4,576	21,875	20,065
461H	25,197	20,647	5,522	6,297	30,701	26,939
464H	11,041	3,758	4,032	4,576	15,072	8,334
327J†	15,869	17,667	3,528	4,004	19,397	21,671
332J	8,178	11,808	3,024	3,432	11,202	15,240
Average	14,314	15,532	3,708	4,211	18,021	19,743
Group IV: Experimental, colostrum 7 days—vitamin capsule 21 days						
307A	32,784	11,080	26,272	28,596	59,056	39,676
311A	15,714	16,128	29,556	32,148	45,270	48,276
292G	14,240	33,424	25,451	27,683	39,691	60,107
294G	7,784	17,872	19,704	21,432	27,488	38,304
451H†	17,843	15,489	26,272	28,576	44,115	44,061
455H	19,980	18,540	31,198	33,934	51,176	52,474
460H	12,160	13,370	32,840	35,720	46,210	47,880
328J†	15,869	17,667	22,988	25,004	38,957	42,671
331J	19,581	19,781	21,246	23,218	40,827	44,163
Average	17,328	18,105	26,170	28,479	43,498	46,629

* The figures in this table represent only the vitamin A intake from colostrum alone in terms of micrograms and does not include the vitamin A administered by capsule.

† These calves nursed the first three days and their carotene and vitamin A intake was estimated by assuming a daily consumption of 1 lb. per 10 lbs. body weight with a potency of the average of the group of cows used in the experiment.

‡ The letter following the calf number indicates the breed, *e.g.*: A = Ayrshire.

It will be noted that there is a rather wide variation in the vitamin A and carotene intake from colostrum during the first 3 days. This arises from the fact that in addition to some variation in weight of the calves there was considerable variation in the potency of the colostrum produced by the individual cows. As a group, the calves in group II had the highest intake of carotene and vitamin A because the cows in this group produced colostrum that averaged higher in potency than that of the other groups.

It should be pointed out, that in the entire group of 36 calves the lowest vitamin A intake from colostrum during the first 3 days was 47,432 International Units and the average was 96,300, or a minimum daily intake of 15,610 and an average of 32,100 International Units. Considering that those calves were all born during the winter feeding period, the majority of them during January, February and March, the amount of vitamin A supplied to the calf through the colostrum is impressive.

From the fourth to the seventh day the intake of vitamin A and carotene from mixed colostrum in Groups II and IV are quite similar; also, the intake from mixed Holstein milk in groups I and III are much alike. Since the same potency figures were used in calculating the intake from colostrum in the former and from milk in the latter groups, the differences recorded arise from differences in the weight of the calves. Although the method of calculating these intakes is subject to criticism because of the lack of preciseness, the use of mixed colostrum and mixed milk for feeding during this period would tend to eliminate much of the irregularity that existed between the individual calves during the first 3 days when each calf was fed colostrum from its own dam.

Table 2 shows the total vitamin A intake of the individual calves and the average for each of the groups in International Units. This unitage was arrived at as follows: Micrograms of vitamin A (table 1) × 4 + micrograms of carotene (table 1) × 1.66 + 10,000 International Units daily from the capsule fed to groups III and IV.

It will be noted that the difference in the average total intake between groups I and III is almost exactly the amount administered by capsule (70,000 I.U.). A similar difference should have occurred between groups II and IV had the dams produced colostrum of comparable potency; however, as mentioned previously, the cows represented in group II produced colostrum having a higher average potency than those of the other groups. This materially reduced the difference in intake between the calves of groups II and IV.

The blood picture. The vitamin A, carotene, and ascorbic acid changes in the blood plasma of the individual calves are presented in tables 3, 4, and 5 and summarized in table 6.

It will be noted that the calves had extremely low levels of vitamin A in the blood plasma at birth. This substantiates the finding of Lundquist and

TABLE 2

Total vitamin A intake of the calves during the first 7 days of the experiment

Results expressed as International Units*

Calf No.	1st 3 days	4th to 7th days inclusive	Total for 7-day period
Group I: Control, colostrum 3 days + placebo capsule 21 days			
310A	78,561	23,755	102,316
314A	77,444	19,605	97,049
291G	90,922	19,605	110,527
298G	70,160	17,888	88,048
449H	97,350	23,755	121,105
459H	84,559	26,004	110,563
463H	93,733	29,613	123,346
330J	97,563	19,605	117,169
335J	52,288	17,888	70,176
Average	82,508	21,969	104,478
Group II: Experimental, colostrum 7 days + placebo capsule 21 days			
309A	193,913	190,893	386,806
313A	126,064	152,748	278,812
290G	90,922	152,748	243,670
296G	103,750	114,536	218,286
450H	97,350	147,942	245,292
457H	89,753	190,893	260,646
465H	134,033	190,893	324,926
329J	131,510	152,748	284,258
334J	113,636	133,625	247,261
Average	117,711	159,666	278,775
Group III: Experimental, colostrum 3 days + vitamin capsule 21 days			
308A	105,824	63,755	169,579
312A	77,432	59,605	137,037
293G	120,712	61,527	182,239
295G	136,326	51,877	188,203
448H	127,350	63,755	191,105
461H	165,121	72,574	237,695
464H	80,423	63,755	144,178
327J	122,921	58,602	181,526
332J	82,392	57,888	140,280
Average	113,167	61,482	174,649
Group IV: Experimental, colostrum 7 days + vitamin capsule 21 days			
307A	179,602	192,748	312,350
311A	119,736	211,804	331,540
292G	142,667	187,942	330,609
294G	90,923	154,536	245,459
451H	127,350	197,748	325,098
455H	140,820	221,349	362,169
460H	100,923	230,893	331,816
328J	122,921	173,615	296,536
331J	141,292	163,681	314,973
Average	129,292	192,695	322,277

* International Units of Vitamin A reported in this table were obtained as follows: (a) Micrograms of vitamin A (Table I) × 4.0; (b) Micrograms of Carotene (Table I) × 1.66; (c) 10,000 I.U. daily from the vitamin capsule in groups III and IV; (a) + (b) + (c) = total International Units.

TABLE 3

The effect of colostrum and vitamin capsule feeding on the blood-plasma vitamin A of calves

Results expressed in micrograms per 100 ml. plasma

Calf No.	Age of calf in days							
	0	3	5	7	9	11	14	21
Group I: Control, colostrum 3 days + placebo capsule 21 days								
310A	6.1	19.6	14.5	18.3	15.7	20.0	15.5	16.8
314A	6.2	27.8	23.2	12.8	15.9	15.5	15.9	11.2
291G	3.5	18.0	14.4	14.2	14.4	13.9		
298G	5.6	10.1	9.6	7.0	7.1	9.1	7.2	5.4
449H	4.8	16.3	10.1		14.2	10.1	10.7	
459H	4.3	12.9	9.3	12.2	10.6	9.9	11.6	9.4
463H	3.5	12.8	13.0	13.2	14.6	13.3	11.4	9.1
330J	1.7	22.1	9.5	8.4	9.3	9.7	12.4	8.1
335J	4.4	18.9	13.1	10.9	9.2	15.2	13.7	8.5
Group II: Experimental, colostrum 7 days + placebo capsule 21 days								
309A	6.3	28.8	19.9	24.1	14.8	14.7	14.3	12.8
313A	6.3	5.3	13.1	20.4	12.1	11.2	15.7	9.5
290G	2.1	13.7	20.8	12.7	19.9	11.8	9.5	
296G	1.9	10.1	13.4	9.7	9.4	8.6	9.9	11.8
450H	9.4	18.3	15.2	16.0	12.6	9.9	12.3	
457H	4.9	25.8	22.9	22.2	16.4	13.5	10.8	14.8
465H	5.0	13.6	20.9	18.1	21.3	18.1	13.2	11.9
329J	7.3	15.8	18.0	16.5	16.7	13.2	12.0	
334J	4.5	20.4	25.7	31.1	18.2	17.3	13.9	14.2
Group III: Experimental, colostrum 3 days + vitamin capsule 21 days								
308A	3.7	19.3	17.2	17.0	16.8	22.3	20.0	21.6
312A	3.8	12.6	12.7	11.9	15.9	13.3	17.2	12.8
293G	3.1	15.0	14.1	12.8	10.4	9.4	15.3	
295G	2.3	15.9	12.8	10.8	12.0	11.2	15.7	13.1
448H	6.5	14.3		13.9	11.7	16.6	10.1	
461H	3.9	16.8	11.1	10.6	11.0	12.8	12.2	10.6
464H	4.3	14.1	10.3	9.3	12.5	7.6	12.6	8.7
327J	5.9	16.0	13.5	18.2	18.5	18.7	10.4	
332J	4.4	17.3	9.0	11.4	8.3	11.8	14.6	9.3
Group IV: Experimental, colostrum 7 days + vitamin capsule 21 days								
307A	3.7	23.3	27.7	27.9	20.1	19.8	19.2	14.1
311A	6.2	26.1	20.8	31.3	21.9	20.3	22.5	17.5
292G	4.4	18.7	15.1	13.8	14.4	10.7	16.9	
294G	3.4	15.4	13.0	19.3	17.7	9.2	11.1	12.1
451H	9.0	16.8	21.5	24.3	15.1	12.2	10.6	
455H	2.2	20.4	24.7	27.5	21.5	17.2	14.6	
460H	5.1	16.7	16.1	15.3	10.3	11.8	10.5	13.0
328J	2.2	16.2	13.9	16.5	13.4	18.6	14.9	
331J	2.1	15.7	17.8	20.1	15.3	12.0	17.7	10.9

Phillips (8) and Moore and Berry (10). The ingestion of colostrum results in a rapid increase in the amount of this vitamin in the blood as others (8, 10) have noted. Similar changes are noted in the case of carotene.

The results reported in tables 3, 4, and 6 also show the effects of extending the colostrum feeding period to 7 days on the blood-plasma vitamin A

TABLE 4

The effects of colostrum and vitamin capsule feeding on the blood plasma carotene of calves

Results expressed in micrograms per 100 ml. plasma

Calf No.	Age of calf in days							
	0	3	5	7	9	11	14	21
Group I: Control, colostrum 3 days + placebo capsule 21 days								
310A	8.3	22.8	16.9	14.0	14.0	16.9	16.9	42.8
314A	6.9	18.4	16.2	14.0	11.1	16.9	16.9	16.9
291G	6.2	105.5	79.0	75.1	79.0	86.1		
298G	6.2	55.1	51.0	25.8	14.0	25.8	18.4	19.8
449H	8.3	16.9	11.2	10.5	14.0	11.2	8.3	
459H	9.7	22.8	22.8	28.1	25.8	25.1	31.8	21.4
463H	6.9	14.0	11.1	8.3	8.3	11.1	14.0	15.5
330J	14.0	31.8	19.8	14.0	21.3	16.9	19.8	19.8
335J	14.0	28.8	31.0	21.4	15.5	22.1	21.3	19.8
Group II: Experimental, colostrum 7 days + placebo capsule 21 days								
309A	5.5	28.8	41.2	31.8	24.3	28.8	34.9	34.9
313A	5.5	5.5	15.5	11.1	14.0	11.2	14.0	19.8
290G	8.3	67.0	88.0	126.4	101.5	117.8	109.0	
296G	11.2	75.1	71.5	17.7	16.9	22.9	34.9	73.2
450H	16.9	19.8	27.3	37.4	38.1	34.9	37.4	
457H	5.5	25.8	49.3	54.3	49.3	47.7	44.4	38.9
465H	4.2	34.9	54.3	31.8	21.4	22.8	28.8	27.3
329J	11.1	34.9	47.7	69.7	44.5	28.8	25.8	
334J	2.8	44.2	51.0	56.8	61.0	51.0	51.9	57.6
Group III: Experimental, colostrum 3 days + vitamin capsule 21 days								
308A	4.1	16.9	14.0	16.9	19.8	16.9	16.9	28.8
312A	2.8	16.9	25.8	6.9	11.2	6.9	8.3	14.0
293G	11.1	47.5	61.0	57.6	28.8	31.8	54.3	
295G	5.5	28.1	34.9	22.8	25.8	38.1	31.8	52.6
448H	8.3	24.3	39.9	17.7	19.8	12.6	16.9	
461H	0.0	19.8	18.4	15.3	14.0	14.0	12.6	14.0
464H	5.5	5.5	8.3	2.8	7.6	6.9	16.9	6.9
327J	11.2	31.8	22.8	22.1	16.9	25.8	8.3	
332J	13.3	23.2	17.7	14.0	16.9	8.3	8.3	8.3
Group IV: Experimental, colostrum 7 days + vitamin capsule 21 days								
307A	4.1	14.0	19.8	51.0	51.0	42.8	41.2	38.1
311A	3.9	19.8	28.8	28.8	34.9	36.5	39.7	31.8
292G	14.0	71.5	34.9	19.8	54.3	45.2	41.2	
294G	8.3	41.2	31.0	51.0	52.6	25.8	17.7	18.4
451H	6.9	19.1	17.7	22.8	22.8	22.8	19.8	
455H	5.5	22.8	41.2	84.2	65.3	59.3	53.4	
460H	3.5	21.4	30.3	16.9	8.3	8.3	10.4	11.2
328J	5.5	50.9	62.7	57.6	60.1	64.5	54.3	
331J	8.3	36.5	38.1	28.0	42.8	25.8	28.8	19.8

and carotene. When the colostrum feeding period is extended, both the vitamin A and carotene continue to rise, reaching a peak on the 7th day, and the decline following the shift to milk feeding is more gradual. As would be expected when both additional colostrum and a vitamin capsule are provided, the highest level of blood-plasma vitamin A was attained and main-

TABLE 5

The blood plasma ascorbic acid of calves used in this experiment

Results expressed in milligrams per 100 ml. of plasma

Calf No.	Age of calf in days							
	0	3	5	7	9	11	14	21
Group I: Control, colostrum 3 days + placebo capsule 21 days								
310A	0.46	0.37	0.35	0.39	0.35	0.20	0.39	0.37
314A	0.67	0.52	0.50	0.62	0.41	0.50	0.44	0.36
291G	0.82	0.70	0.70	0.72	0.64	0.52	0.23	
298G	1.30	0.71	0.64	0.64	0.48	0.42	0.47	0.29
449H	0.76	0.65	0.33	0.31		0.32	0.23	
459H	1.23	0.60	0.61	0.38	0.47	0.40	0.36	0.42
463H	1.11	0.41	0.52	0.50	0.41	0.47	0.38	0.40
330J	1.23	0.73	0.41	0.49	0.49	0.38	0.33	0.31
335J	1.11	0.38	0.49	0.49	0.49	0.38	0.42	0.36
Group II: Experimental, colostrum 7 days + placebo capsule 21 days								
309A	0.70	0.49	0.53	0.50	0.37	0.44	0.45	0.35
313A	0.66	0.21	0.25	0.39	0.31	0.18	0.16	0.26
290G	0.78	0.44	0.44	0.58	0.47	0.65	0.49	
296G		0.49	0.63	0.38	0.23	0.40	0.42	0.28
450H	0.49	0.43	0.44	0.44	0.35	0.32	0.28	
457H	0.66	0.59	0.53	0.41	0.49	0.35	0.41	0.33
465H	0.88	0.69	0.45	0.50	0.53	0.49	0.48	0.39
329J	1.67	0.73	0.44	0.61	0.48	0.44	0.42	
334J	0.82	0.46	0.39	0.38	0.38	0.36	0.53	0.38
Group III: Experimental, colostrum 3 days + vitamin capsule 21 days								
308A	1.04	0.79	0.77	0.97	0.63	0.67	0.67	0.60
312A	0.64	0.52	0.46	0.50	0.41	0.39	0.38	0.32
293G	0.62	0.70	0.66	0.60	0.43	0.50	0.38	
295G	0.89	0.64	0.58	0.44	0.40	0.38	0.63	0.32
448H	0.70	0.58	0.70	0.57	0.47	0.43	0.29	
461H		0.47	0.50	0.55	0.44	0.40	0.30	0.40
464H	0.97	0.38	0.53	0.62	0.55	0.49	0.52	0.32
327J	0.75	0.44	0.40	0.58	0.36	0.41	0.24	
332J	0.90	0.35	0.59	0.54	0.50	0.41	0.30	0.26
Group IV: Experimental, colostrum 7 days + vitamin capsule 21 days								
307A	0.72	0.61	0.60	0.67	0.54	0.50	0.49	0.40
311A	0.60	0.49	0.46	0.46	0.39	0.51	0.45	0.31
292G	0.70	0.53	0.53		0.46	0.72	0.43	
294G	0.96	0.73	0.67	0.67	0.65	0.74	0.55	0.41
451H	0.93	0.63	0.57	0.53	0.46	0.37	0.36	
455H	1.07	0.70	0.52	0.56	0.42	0.41	0.63	
460H	0.81	0.66	0.55	0.42	0.33	0.26	0.30	0.30
328J	0.56	0.42	0.31	0.52	0.49	0.54	0.39	
331J	0.77	0.48	0.41	0.42	0.41	0.53	0.37	0.24

tained throughout the 21-day period. It is interesting to note that the average vitamin A content of the blood plasma of groups II and III are almost identical on the 21st day. The data in these two groups compare the results obtained when colostrum is fed 7 days (group II) with those obtained when a 10,000 I.U. vitamin A capsule is fed for 21 days (group III).

The data on blood plasma ascorbic acid values substantiates the finding of Lundquist and Phillips (8) that this vitamin is found in the blood of newborn calves at a level considerably higher than that of older calves. The changes following birth, however, were not appreciably affected by the feeding of extra colostrum, a vitamin capsule daily, or a combination of extra colostrum and a vitamin capsule. At the end of the 3-week period the average blood-plasma ascorbic acid was slightly in excess of 0.3 milligrams per 100 ml., which is quite similar to that reported by Lundquist and Phillips (8) for calves over 2 weeks of age.

TABLE 6

Average blood plasma vitamin A, carotene and ascorbic acid values and the average gain in weight of the calves in the experiment, by groups

Group No.	Age of calves in days							
	0	3	5	7	9	11	14	21
	Average blood-plasma vitamin A, γ/100 ml.							
I	4.4	17.6	12.9	12.1	12.3	12.9	12.3	9.8
II	5.3	16.8	18.8	18.9	15.7	13.1	12.4	12.5
III	4.2	15.7	12.6	12.8	13.0	13.7	14.2	12.6
IV	4.2	18.8	18.9	21.7	16.6	14.6	15.3	13.5
	Average blood-plasma carotene, γ/100 ml.							
I	8.9	35.1	28.8	23.5	22.5	25.8	18.5	22.3
II	7.9	37.3	49.5	48.5	41.2	42.3	42.3	41.9
III	6.8	23.8	26.9	19.6	17.8	19.3	19.3	20.8
IV	6.7	33.0	33.8	40.0	43.5	34.0	34.0	23.9
	Average blood-plasma ascorbic acid, mg./100 ml.							
I	0.96	0.56	0.50	0.50	0.47	0.40	0.36	0.36
II	0.83	0.50	0.46	0.47	0.40	0.40	0.40	0.33
III	0.81	0.54	0.57	0.59	0.47	0.45	0.41	0.37
IV	0.79	0.58	0.51	0.53	0.46	0.51	0.44	0.33
	Average gain in weight, lb.							
I				2.6			2.0	
II				4.6			3.6	
III				1.6			2.4	
IV				3.7			1.3	

DISCUSSION

Since, on most dairy farms, the colostrum not nursed by the calf is discarded because it is not marketable, Moore and Berry (10) have suggested that its use in raising calves be studied. Allen (1) has reported that colostrum can be stored (frozen) for a considerable period of time. His work also shows the stored colostrum to be a satisfactory feed for raising dairy calves.

The data reported in this manuscript show the effects of colostrum feeding on the levels of blood-plasma vitamin A, carotene, and ascorbic acid in the dairy calf. When the colostrum feeding period is extended to 7 days, the amount of vitamin A in the blood continues to rise, reaching a peak at 7 days. A comparison of the average data for groups I and II (table 6) show the marked effect of colostrum in this respect. It is felt that this extra colostrum feeding supplies additional vitamin A at a time most critical. The gain-in-weight data in table 6, while not conclusive, strongly indicate beneficial effects on the growth of the calf.

The data from groups II and III compare the effects of 7-day colostrum feeding with the usual (3-day colostrum feeding) scheme plus an additional 10,000 I.U. of vitamin A daily. It should be noted that the extra 4 days of colostrum feeding is an effective substitute for a daily supplement of 10,000 I.U. of vitamin A for the first 21 days. We believe that the evidence is in favor of the colostrum feeding—first, because of the blood vitamin A results which show higher values during the critical early days of the calf's life, and secondly, because the weight-gain data which, though inconclusive, indicate superior growth during the first 2 weeks. It must be remembered in this connection, that the group receiving colostrum for 7 days (group II) had a much higher total solids intake during the first week than did those receiving whole milk after the third day.

A combination of extra colostrum and a vitamin capsule daily (group IV) resulted in the highest level of vitamin A in the blood as would be expected. This may or may not be of significance in the performance of the calf. No data were obtained which indicated superior performance of the calves in this group.

The level of ascorbic acid in the blood was not appreciably changed when extra colostrum, vitamin capsules, or a combination of the two were fed. These data are at variance with those obtained by Lundquist and Phillips (8). It must be remembered, however, that their calves were raised from birth on skim milk, which is probably the reason for the difference in blood-plasma ascorbic acid results.

At the time this experiment was under way a calf in the University Herd was afflicted with navelill. An ascorbic acid analysis of the blood of this calf showed an extremely low value which substantiates the report of Lundquist and Phillips. The feeding of ascorbic acid (approximately 1 gram daily) did not result in the rise in blood-plasma ascorbic acid nor in improvement in the condition of the calf. We do not interpret this as contrary to the results of Lundquist and Phillips, in which the daily injection of 0.5 gm. of ascorbic acid resulted in rapid recovery of calves suffering from navel infections. A more logical interpretation is that the feeding of ascorbic acid is not an effective method for the administration of this vitamin to calves.

During the course of this experiment 15 of the calves showed some evidence of digestive disturbance. These calves had mild to moderate diarrhea.

There were no severe cases. These 15 calves were distributed among the groups as follows: group I, 3; group II, 3; group III, 5; and group IV, 4. Thus 7 were in the groups receiving colostrum for the 7-day period and 8 were in the groups receiving colostrum for the 3-day period. One interesting, and perhaps significant, difference was noted. The calves that received colostrum for 7 days developed diarrhea when they were from 9 to 11 days of age. It is believed that the abrupt change from colostrum to mixed-whole milk may have been a contributing cause. During the time the calves were fed colostrum the calves were housed in warm box stalls and handled by a special caretaker. At the end of their colostrum feeding they were moved to the calf barn where the temperature was considerably lower. A change in caretakers accompanied the move. These conditions may have also contributed to the occurrence of diarrhea. Diarrhea appeared in the calves receiving colostrum for 3 days, when they were from 4 to 11 days of age. This condition developed in 5 of the 8 calves when they were from 4 to 7 days of age. None of the calves in this experiment had scours of the so-called infectious or "white" type.

In 11 of the 15 calves that had diarrhea there was a simultaneous drop in blood-plasma vitamin A and carotene. When the diarrhea cleared up, the blood plasma vitamin A and carotene increased, but did not reach the previous level again; this was to be expected since the general trend in all of the calves was downward. To illustrate: The average level of vitamin A in the blood plasma of these 11 calves preceding the onset of diarrhea was 17.0 micrograms per 100 ml.; during diarrhea, 11.0; and after recovery 14.8 micrograms per 100 ml.

Moore and Berry (10) have pointed out that a blood-plasma response to colostrum feeding is essential to the general health of the newborn calf. His findings, that calves which failed to respond to colostrum were not likely to survive, is substantiated, at least in part, by the performance of calf No. 289G in this experiment. Although this calf had an intake of over 70,000 I.U. of vitamin A during its first 3 days, its blood-plasma vitamin A did not go above 10 micrograms per 100 ml. This calf never performed satisfactorily, and died at approximately 3 months of age.

During the course of this experiment over 650 pounds of colostrum were fed which would otherwise have been wasted. This was not all of the colostrum which was saved during the course of the experiment, but it serves to illustrate the economy of the procedure. Every pound of colostrum saved and used in this way not only is beneficial to the calves being raised but replaces a pound of marketable milk. Under practical dairy-farm conditions it may not be convenient to refrigerate and hold colostrum for feeding according to the plan employed in this experiment. Other plans for its use can no doubt be worked out and adapted to individual dairy farms. A practical experiment to study this point is being planned.

SUMMARY

An experiment designed to study some of the physiological effects of extending the colostrum feeding period was carried out on 36 dairy calves.

In substantiation of the reports of others it was found that calves are born with low reserves of vitamin A but with ascorbic acid reserves considerably above that of later life, as measured by the level of these constituents in the blood plasma.

Blood plasma vitamin A increases rapidly following the ingestion of colostrum. In the calves receiving no other supplement, in this experiment, the average increase amounted to 12.4 micrograms per 100 ml. plasma in the first 3 days. This increase resulted from an average intake of 99,614 International Units of vitamin A from colostrum during the first 3 days.

When the period of colostrum feeding was extended to 7 days the blood-plasma vitamin A continued to increase, reaching a peak on the seventh day. The levels reached on the 7th day averaged 18.9 micrograms per 100 ml. plasma, which was 6.8 micrograms above that of 7-day-old calves which had received colostrum for 3 days.

Calves which received colostrum for 7 days had blood-plasma vitamin A values which averaged almost identically the same at 21 days of age as that of those which received colostrum for 3 days plus a 10,000 I.U. vitamin A capsule daily for the first 21 days.

None of the variations in feeding procedure employed in this experiment appreciably influenced the level of ascorbic acid in the blood plasma.

None of the calves receiving colostrum for 7 days showed any signs of digestive disturbance during the period of colostrum feeding.

Weight records indicate more rapid gains in calves receiving additional colostrum.

The economy of using the colostrum not nursed by the calf for calf feeding purposes is pointed out.

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FORMIC ACID CONTENT OF MILK HEATED TO HIGH TEMPERATURES AS DETERMINED BY THE DISTILLATION PROCEDURE[1]

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In a former study (3), determinations for formic acid were made on the steam distillate from skim milk heated to 116° C. for two hours, using the A.O.A.C. distillation method (1). Results obtained revealed that 80–85 per cent of the total volatile acidity in the distillate was recovered as formic acid when a 20 per cent allowance was made for the incompleteness of recovery by the method used. Thus, these findings indicate formic acid to constitute a large proportion of the volatile acids created when milk is heated at temperatures sufficient to produce lactose decomposition. However, they do not answer the question as to the relative proportion of formic acid to total acidity changes in the milk itself.

Results obtained in two previous papers pertaining to the subject of heat-produced acid in milk (2, 3) reveal the following pertinent facts: (a) lactic acid constitutes not more than 5 per cent of the total titrable acidity produced in milk by heat; (b) the molar destruction of lactose was three times as great as the molar production of titrable acidity expressed as lactic acid; (c) heating of milk at high temperatures creates appreciable quantities of steam volatile acidity, a large share of which is apparently formic acid; (d) the A.O.A.C. method of measuring formic acid does not give quantitative recovery of the acid from an aqueous solution and a correction factor is necessary in order to obtain at least fairly accurate results. A review of earlier research dealing with the production of acid in milk by heat is also presented in these papers.

In view of the fact that the earlier work (3) dealt with formic acid in the steam distillate from heated milk, it appeared desirable to obtain results in which formic acid was determined directly upon the heated milk. Such results serve as the basis for this paper.

PROCEDURE

The formic acid procedure was essentially that recommended by the A.O.A.C. for fruit juices, sirups and jams (1). A 250-ml. sample of milk was used and the volume increased to 300 ml. with 10 ml. distilled water and 40 ml. N H_2SO_4. A small amount of paraffin was added to prevent foaming

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and the sample steam distilled with the vapor passing through 200 ml. of one per cent $BaCO_3$ solution, through a condenser, and into a liter flask. The distillation was continued until one liter of condensate was obtained. The $BaCO_3$ solution containing the volatile acids was then reduced to 100 ml., filtered, and the filtrate treated with sodium acetate, hydrochloric acid and mercuric chloride. The precipitated mercury salt of formic acid was filtered, dried, weighed, and calculated to formic acid.

Heating of the milk was conducted as previously described (2), by using 500-ml. samples in sealed "Cenco" No. 2 cans and heating in the autoclave. The samples were shaken at 30-minute intervals during the heating period.

Titrable acidity of the milk was determined electrometrically, using a glass electrode and a Beckman pH meter and titrating to pH 8.3, using 0.1 N NaOH.

TABLE 1

Recovery of added formic acid from milk by the distillation procedure

Trial No.	Formic acid added	Formic acid recovered	
		Amount	Per cent
	mg./250 ml.	*mg./250 ml.*	
1 a	20.3	14.1	69.5
2 a	20.5	14.4	70.2
2 b	20.5	14.6	71.2
3 a	41.4	29.2	70.5
3 b	41.4	25.5	61.6
4 a	82.8	57.3	69.2
4 b	82.8	53.6	64.7
5 a	82.8	53.0	64.0
5 b	82.8	51.8	62.6
6 a	82.1	51.6	62.8
6 b	82.1	49.3	60.0
7 a	103.2	72.8	70.5
7 b	103.2	70.4	68.2
Avg.			66.5

RESULTS

Formic acid in unheated milk. Before subjecting heated milk directly to the formic acid procedure, trials were conducted with unheated (raw) milk. This was done to ascertain whether or not the heating of the milk during the formic acid determination would, in itself, create appreciable quantities of formic acid. Results revealed that, following correction for the reagent blank, formic acid results ranged from approximately 0.3 to 0.5 mg./250 ml. of milk, a negligible amount. Reagent blanks for the determinations were equivalent to less than 0.3 mg. of formic acid.

Recovery of added formic acid from milk. Since the previous study (3) had shown that complete recovery of formic acid was not obtained from an aqueous solution, it appeared desirable to ascertain the recovery of formic acid from milk. Results of such determinations are presented in table 1.

These data reveal that the distillation method recovered from approximately 60 to 70 per cent of the added formic acid with an average recovery of 66.5 per cent. In seven of the thirteen trials the recovery was more than 68 per cent, whereas in the other six trials the recovery was less than 65 per cent. Efforts to determine the cause for these variations were unsuccessful. One such effort involved the titration of the condensate to determine if appreciable quantities of acid were unremoved by the $BaCO_3$ trap. The results of these titrations did reveal that a slight and variable amount of excess acidity was found in the condensate. However, the amount in general was small and showed no relationship either with the quantity of formic acid in the sample or with the per cent recovery. Since, in later trials involving heated milk, the concentrate acidities were of the same magnitude, no correction is made to compensate for losses to this source.

TABLE 2

Formic acid produced by heating milk at 116° for one- and two-hour periods as determined by the distillation procedure

Heating period	Titrable acidity increase as formic	Formic acid measured	Corrected formic acid produced*	Proportion of total acid which is formic
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	%
One hour				
Lot 1	20.9	6.5	9.8	46.9
Lot 2	20.5	6.9	10.4	50.7
Lot 3	18.9	6.4	9.6	50.8
Avg.	20.1	6.6	9.9	49.2
Two hours				
Lot 1	38.6	13.6	20.4	52.8
Lot 2	35.8	14.4	21.6	60.3
Lot 3	40.3	15.6	23.4	58.1
Avg.	38.2	14.5	21.8	57.1

* Corrected on basis that only 66.5 per cent of formic acid in milk recovered by method used.

On the basis of the findings in table 1, it appears that the distillation method may be used at least fairly satisfactorily in determining the formic acid content of heated milk by assuming that recovery is only 66.5 per cent. This correction should give an accuracy within 5 per cent.

Formic acid in heated milk. Commercial homogenized, pasteurized milk was heated in the autoclave for one- and two-hour periods at 116° C. Titrable acidities were measured before and after heating and the increase in acidity expressed as formic acid. Formic acid determinations were made in 250-ml. quantities. Results are shown in table 2.

These data reveal that the formic acid actually recovered from the one-hour sample amounted to 32.8 per cent (6.6 mg./100 ml.) of the total titrable acidity, whereas the recovery was 37.9 per cent (14.5 mg./100 ml.) for the two-hour sample. By applying the correction, formic acid is found to constitute, on the average, 49.2 and 57.1 per cent of the total heat-produced acidity for the one-hour and two-hour samples, respectively.

DISCUSSION

Results presented herein have several points in common with those in the earlier paper on this subject (3). The data again reveal the inadequacy of the distillation procedure to permit a direct, highly quantitative measurement of formic acid in milk. It is true that by use of a large corrective factor, results are obtained which appear satisfactory and which, for the time being, must be accepted as at least fairly accurate. However, a more sensitive and direct method of formic acid determination is desirable if a thorough study is to be made of the possible rôle of this acid in influencing the quality of dairy products processed at high temperatures.

The results in this study also again reveal the rather high concentration of formic acid produced by heat. However, the proportion of formic acid to total titrable acid in the milk itself is decidedly less than when the distillate was involved and when only volatile acids were concerned.

The difference between the titrable acidity results and the formic acid results may not represent only changes in heat-produced acid. As was pointed out earlier (4), various changes occur when milk is heated for such temperatures and times as used in this study, changes which may affect the titrable acidity although not necessarily involving acid production. It is interesting to note that the proportion of formic to titrable acidity increase is greater in the sample heated for two hours than in the sample heated for one hour, *viz.*, 49.2 per cent to 57.1 per cent. This may mean that as the heating time progresses there is (a) relatively greater production of formic acid than other acids, or (b) that there is less of these so-called "non-acid" changes. The previous work (4) does indicate that salt changes may occur early during the heating period and these changes may directly affect the titer.

SUMMARY

Formic acid determinations by the distillation method were made on milk to which known amounts of formic acid were added and on milk which had been heated for one- and two-hour periods at 116° C.

From 60 to 71 per cent of the added formic acid was recovered by the method used with an average recovery of 66.5 per cent.

Milk heated for one hour averaged 9.9 mg. of formic acid per 100 ml. of milk when the retention correction was used. This represented approximately 49 per cent of the total titrable acidity increase. In contrast, milk heated for two hours contained a corrected average of 21.8 mg. formic acid per 100 ml. of milk, or an amount equivalent to approximately 57 per cent of the total titrable acidity increase.

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LACTIC ACID IN DAIRY PRODUCTS. IV. EFFECT OF STORAGE OF EVAPORATED MILK ON LACTIC AND FORMIC ACID PRODUCTION, ON pH, AND ON ACIDITY AND FORMOL TITRATIONS[1]

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Preceding papers in this series (4, 8) have dealt with such factors as (a) microbiological changes and (b) high temperature heat treatment as they affect the lactic acid content of milk and milk products. One other important factor in this connection is that of storage, and it is the purpose of this paper to present information on this subject as it pertains to evaporated milk.

In connection with the lactic acid studies it appeared desirable also to ascertain the extent of other changes in evaporated milk which result from storage. Consequently, determinations were made on fresh and stored samples for formic acid, pH, titrable acidity, and formol titration.

PROCEDURE

Fresh evaporated milk was obtained directly from the manufacturers. Storage was at the specified temperatures with the samples being shaken at weekly intervals to prevent excessive fat separation and salt precipitation.

General methods of analysis were those used in previous studies (3, 4, 6, 7) with the exception that certain slight adaptations were necessary in order that the methods could be applied to the evaporated product.

The Hillig procedure (3, 9, 10) was used for lactic acid determinations. Some difficulty was experienced with the extraction of those samples stored for a considerable period of time at 120° F. due to the formation of a stable form which was carried over into the extraction flask. This difficulty was overcome by modifying the proportions of the precipitants used in preparing the serum for extraction, *i.e.*, using 9 ml. instead of 6 ml. of N sulfuric acid and 10 ml. instead of 5 ml. of 20 per cent phosphotungstic acid.

Formic acid was measured on a 250-ml. reconstituted sample (125 ml. evaporated milk and 125 ml. water) using essentially the A.O.A.C. method (1) described previously (5, 6). On the basis of earlier findings (6), correction of the results was made on the assumption that the method recovers only 66.5 per cent of the formic acid present.

pH determinations and titrations were made electrometrically, also on

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reconstituted samples. As in the previous study (7) titrations consisted of the following:

Normal—Untreated sample.

Oxalate—Sample treated with 2 ml. of a neutral saturated potassium oxalate solution per 50 ml. sample, prior to titration.

Formol—Addition of 5 ml. neutralized formalin per 50 ml. sample after titration, and again titrating to pH 8.3.

Formol oxalate—Formol titration of a sample treated with oxalate.

RESULTS

Effects of storage at different temperatures on lactic acid production. The first trials conducted in this study concerned the effect of storage period and temperature on the lactic acid content of evaporated milk. Samples of fresh evaporated milk, differing somewhat in lactic acid, were obtained through the courtesy of the Evaporated Milk Association from two different manufacturers. Samples were stored at 35°, 70°, 100°, and 120° F. with analyses being made at monthly intervals. Results are shown in table 1.

TABLE 1

Effect of temperature and time of storage of evaporated milk on the lactic acid content

Temperature of storage (°F.)	Month of storage					
	0	1	2	3	5	7
	Lactic acid (mg./100 gm. evaporated milk)					
Series 1						
35	17.0		16.0	17.0	16.6	18.6
70			18.6	18.6	18.6	18.4
100		17.2	18.0	18.6	20.0	22.0
120		17.2	30.0	30.0	34.0	*
Series 2						
35	10.0	..	9.6	9.8	10.4	10.6
70			9.6	10.4	10.6	12.2
100		10.4	11.5	11.6	11.6	12.2
120		10.2	18.6	18.4	22.2	*

* Sample deteriorated to such an extent that analysis could not be made.

The results reveal that, in general, there is a tendency for the lactic acid content of the evaporated milk to increase in storage. However, increases at temperatures of 35°, 70°, and 100° F. for series 2, and at 35°, and 70° F. for series 1 are so slight as to be within experimental error. In both series, the storage at 120° F. produced appreciable quantities of lactic acid. However, these samples had deteriorated so greatly by the end of the second month that they were no longer of marketable quality.

Lactic and formic acid relationships. In additional trials, fresh samples of evaporated milk were again obtained from two different processing plants. These samples were stored at 100° F. for seven months. At monthly intervals the following determinations were made: lactic acid, formic acid, pH, titrable acidity before and after oxalation, and formol titration before and after oxalation. Results for the lactic and formic acid determinations are shown in figure 1. Formic acid is expressed directly as measured by the A.O.A.C. method and also as corrected on the basis that recovery by the method is only 66.5 per cent. This is in accordance with earlier findings for milk (6).

This figure reveals again that storage at 100° F. for the length of this experiment produced negligible increases in lactic acid. The average lactic acid content of the samples was 11 mg. per 100 gm. and this value had changed to slightly over 12 mg. per 100 gm. by the end of the storage period.

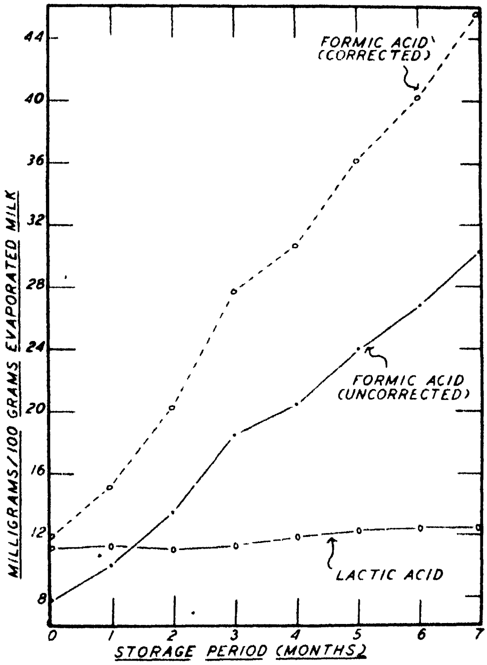

Fig. 1. Lactic and formic acid changes in evaporated milk stored for seven months at 100° F.

The formic acid findings are in contrast to those for lactic acid in that they indicate a marked change during storage. The uncorrected formic acid results reveal an increase in formic acid from about 7.9 to 30.1 mg. per 100 gm. evaporated milk, whereas the corrected formic acid results show a change from about 11.9 to 44.8 mg. per 100 gm. evaporated milk.

Titrable acidity and pH. The effect of storing the evaporated milk on the acidity and formol titrations of normal and oxalated samples is shown in figure 2.

The results portrayed in this graph reveal that increases in titrable acidity occur with storage, that the increase is greater when the measurement is on the oxalated sample, and that the formol titration undergoes no appreciable change.

The difference between the titration curves for the normal and for the oxalated samples is striking. Whereas the titers of the normal evaporated milk reveal a change during storage equivalent to about 8.7 ml. 0.1 N NaOH per 100 gm. milk, the titers of the oxalated sample show a change equivalent to 17.7 ml. 0.1 N NaOH per 100 gm. Furthermore, the titrable acidity curve levels off at about the fifth month of storage, but the oxalate curve continues upward throughout the entire storage period.

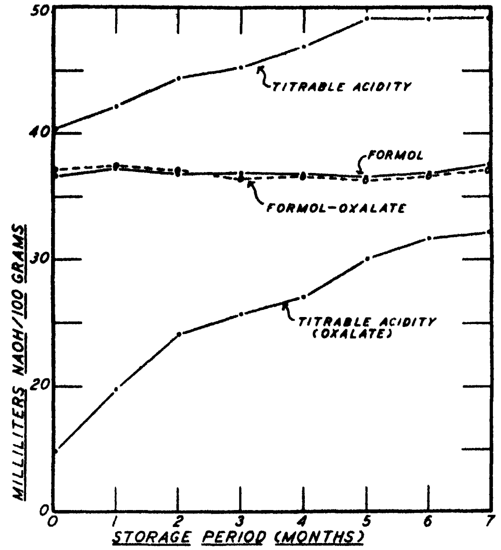

FIG. 2. Titration changes in evaporated milk stored for seven months at 100° F.

pH results on these samples are portrayed in figure 3. In general, these curves are in keeping with the titration results in figure 2. For example, the normal and oxalate curves exhibit a definite decrease in pH with prolongation of the storage period, the decrease being more marked in the case of the oxalated samples. However, the formol pH curves do not correlate completely with the respective titration curves. The pH curve for the formol titration of the normal sample indicate a slight but definite decrease, whereas the titration values themselves showed no distinguishable trend. Furthermore, although the pH curves for the formol-normal and formol-oxalate samples are at definitely different levels, the two respective titration curves were practically identical; *i.e.*, both samples required practically the same quantity of NaOH to adjust the pH to 8.3.

Titrable acidity and formic acid relationship. The fact that formic acid increases were so pronounced as a result of storage, raised the question as to

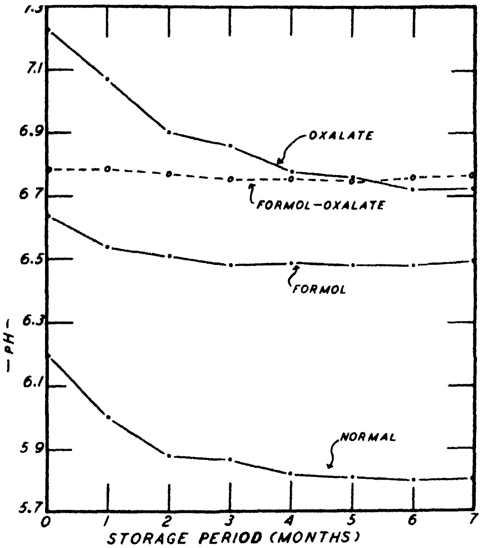

Fig. 3. pH changes in evaporated milk stored for seven months at 100° F.

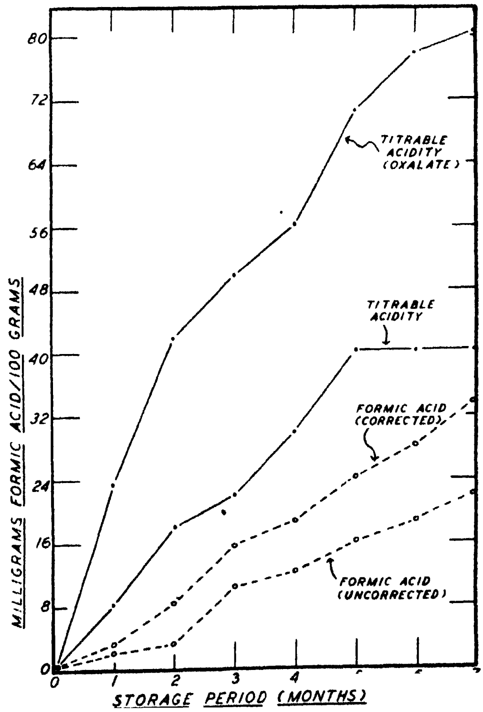

Fig. 4. Increases in acidity and formic acid in evaporated milk during seven months of storage at 100° F. (All values expressed as formic acid.)

the proportion of the titrable acidity which may be attributed to this acid. To throw light on the question, a comparison was made between the formic acid and the titrable acidity increases. The results of this comparison are shown in figure 4 in which all values are expressed as formic acid.

The curves representing changes in the titrable acidity and those representing increases in formic acid possess similar slopes for the first five months, but with the titrable acidity curve somewhat steeper. Following this period, however, the formic acid curves continued upward whereas the titrable acidity curve leveled off abruptly. At the close of the storage, the increase in formic acid amounted to 22.4 mg. per 100 gm. (uncorrected) and 33.8 mg. per 100 gm. (corrected) whereas the titrable acidity increase was equivalent to 40.4 mg. per 100 gm. evaporated milk.

A more drastic difference results when the formic acid to total acid comparison is made using the oxalate acidity values. In this case, the increase in acidity when expressed as formic acid was 80.6 mg. per 100 gm. evaporated milk.

DISCUSSION

The results obtained in this study reveal that formation of lactic acid in evaporated milk on storage is of no great significance. Even under extreme adverse conditions of storage, the amount of lactic acid formed would be so slight as to not invalidate any decision that might utilize the lactic acid content as being indicative of the quality of the original raw milk.

An interesting aspect of this study is the production of formic acid in the stored evaporated milk. In fact, it is interesting to note that even the fresh evaporated milk contains appreciable amounts of this acid, the amount averaging about 7.9 mg. per 100 gm. and 11.8 mg. per 100 gm. respectively on the uncorrected and corrected bases. However, the presence of formic acid in evaporated milk is not entirely unexpected since this acid is the result of sugar decomposition and since the heat treatment to which evaporated milk is subjected is sufficient to produce such decomposition. The marked increase in formic acid during the storage of the evaporated milk may be taken to indicate further lactose destruction.

In regard to these formic acid results, it should be emphasized that the method used is not entirely satisfactory and is doubtless lacking in sensitivity. The use of such a large factor to correct for retention in the distillation process is naturally not desirable. However, even with the shortcomings of this method, the results obtained definitely point the way to the fact that formic acid is present in milk products subjected to high temperatures and that this acid may be related to certain flavor changes and to color production in these products.

pH and titrable acidity changes which occur in evaporated milk on storage are to be expected on the basis of the formic acid findings; *i.e.*, the pH decreases and the titrable acidity increases. The decrease in pH from 6.2 to 5.8 is in accordance with the findings of Doan and Josephson (2). The pH curve exhibited a definite leveling off after about the fourth month of storage and the titrable acidity ceased to increase after the fifth month.

The effect of the milk salts on titrable acidities is indicated by the results obtained. It is known that storage of evaporated milk results in the formation of salt crystals which precipitate from the milk. Such crystals, in small quantities, were observed in these samples. Possibly the abrupt leveling off of the titrable acidity curve after the five-month period is a result of salt rearrangements and precipitation, these changes compensating for the increase in acidity created by formic and other acids. The difference between the oxalate curve and the titrable acidity curve further emphasizes the salt effect. Since the oxalate curve exhibited a greater acidity change than the normal titration curve and, furthermore, since it revealed a steady acidity increase throughout storage whereas the normal titration curve did not, the possibility exists that the oxalate treatment of evaporated milk prior to titration may afford a superior means of measuring storage changes in acidity than is obtained by the standard titration.

Relative to the titration and pH results, it should be stressed that they need not apply precisely to all evaporated milk. In this study, these results are representative of storage changes in series of fresh evaporated milk obtained from two processors. However, differences in the quality of the original milk, differences in processing methods, or differences in the amount and kind of stabilizing salts may definitely influence the results obtained.

On the basis of the results obtained in this study, the formic acid produced in evaporated milk during the seven-month storage at 100° F. constitutes about 71.3 per cent of the total acidity as determined by titration when correction is made for retention in the distillation process. However, as indicated earlier in this discussion, to arrive at a strictly accurate value in this connection one is faced with two complications: (a) that the formic acid method used leaves considerable to be desired from the standpoint of extreme accuracy, and (b) that the titration procedure itself does not necessarily assure one of a true measure of total acid production. For example, one might prefer to use the formic acid values as obtained directly by the distillation procedure and without resorting to any further correction. In this case, the proportion of formic acid to total acid would be decidedly less, viz., 55.4 per cent as contrasted to 71.3 per cent. In addition, perhaps the acidity increase as obtained in the oxalate sample may be used to represent the true total acid change. Again, if such were done, the proportion of formic acid to total acid would be markedly less than if the standard titration results served as the basis of comparison. Additional studies on this subject are necessary before final conclusions may be drawn.

SUMMARY AND CONCLUSIONS

Increases in lactic acid in evaporated milk stored at 35°, 70° and 100° F. for seven months averaged 1.1, 1.7 and 2.4 mg. per 100 gm. respectively.

These small increases are, for all practical purposes, insignificant. However, storage of the milk at 120° F. for six months resulted in an average lactic acid increase of 14.6 mg. per 100 gms., but at this time the milk had deteriorated far beyond the marketable stage.

In an additional experiment in which evaporated milk was stored at 100° F. for seven months, lactic acid changes were again insignificant, but marked increases occurred in titrable acidity and formic acid, accompanied by a decrease in pH. Titrations of oxalated samples indicated much larger acidity increases than were revealed by the standard titration procedure. Formol titrations were essentially unaffected by storage.

Formic acid constitutes a significant portion of the total acid produced in evaporated milk as a result of storage. Under the conditions of this experiment, and when the recovery of formic acid is corrected for retention, the increase in formic acid during storage was equivalent to 71.3 per cent of the total acidity increase as determined by titration.

Limitations of the methods and possible applications of the findings are discussed.

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INFLUENCE OF HOMOGENIZATION OF FAT ON HAIRCOAT OF DAIRY CALVES

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Many reports have been made concerning the value of filled milks (skim milk and an unnatural fat) for rearing young calves. In studies on infant nutrition the importance of the size of the fat particles is often stressed but little actual evidence has been provided to support this theory. In most calf-feeding trials no attention has been given to the physical form of the fat. In one trial (1) the authors mention a characteristic loss of hair but do not associate it with the physical condition of the fat.

A year ago we started feeding skim milk and soybean oil (hereafter referred to as ration one) to calves on the fifth day after birth. Skim milk was warmed and fed with a nipple pail at the rate of 7.5 pounds daily per hundred pounds liveweight. The oil was given by syringe after the milk was fed. We had expected to give an amount of oil equivalent to 3.3 per cent of the skim milk but were forced to reduce the amount to approximately 2 per cent to avoid nutritional scours. Alfalfa hay and a grain mixture consisting of ground corn, rolled hulled oats, bran, and linseed meal were fed, *ad libitum*. The grain mixture contained 1 per cent salt, 1 per cent bone meal, and 0.05 per cent irradiated yeast. Each calf received a fish oil concentrate thrice weekly equivalent to 8500 I.U. of vitamin A per day.

By the time these calves were two weeks old the hair on the inside of the thighs began to fall out (see figure). In most cases only the hair over the perineal region was affected, although occasionally the hair about the muzzle also dropped out. A heavy "dandruff" developed over the rest of the body but the hair appeared fairly healthy. Scrapings from the perineal region which were examined by the Veterinary Pathology Department showed no primary infection or parasitic organisms, nor was the alopecia due to soiling of the hair with fecal material.

The first disturbance to be noted in the perineal region was a cracking of the skin. Within a few days the outer epidermal layers, together with the hair, would die and slough off. Although this condition produced some discomfort, the calves showed no marked tendency to rub or lick the affected area. The skin remained free of hair until the calves began eating hay or grain. Within a few weeks after their rumens started to function the hair began to grow again even though the oil was still fed in an unhomogenized

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form. Calves started on skim milk and oil after they had begun to ruminate never developed the condition just described. Other oils, including butter oil, had the same effect on the haircoat as soybean oil.

A second group of calves was fed on skim milk into which soybean oil was homogenized (hereafter referred to as ration two). Twice a day the desired amount of oil was homogenized into about a pint of warm separated skim milk with a hand homogenizer, then made up to the required volume and fed with a nipple pail. No calf in this group lost any hair prematurely.

A calf which previously had been fed on skim milk and soybean oil (ration one) was fed with this group on ration two. It was kept muzzled so it could get no roughage or grain. After seven days on ration one the hair began falling out. When given ration two the hair again started to grow. Ration two was then replaced by ration one and within ten days the upper layers of the skin in the perineal region began cracking and showing a very definite metabolic disturbance.

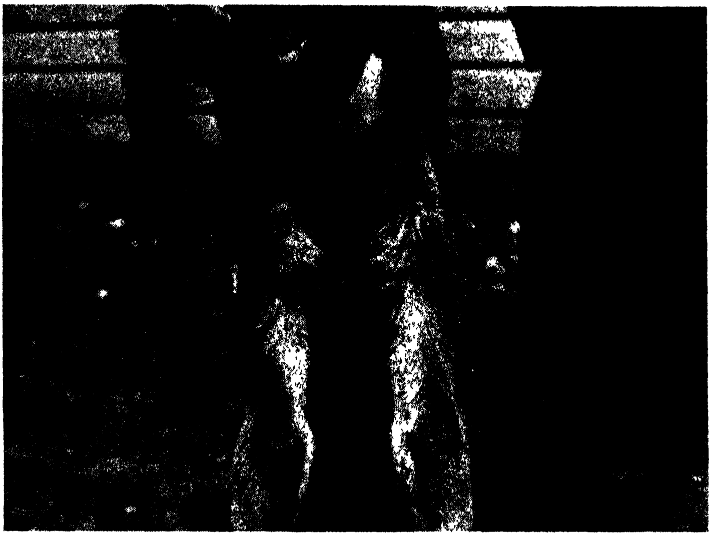

FIG. 1. Effect of feeding unhomogenized oil on haircoat of young dairy calves.

We are unable to offer any explanation for the condition just described. It should be emphasized that the alopecia we have described has never occurred with calves fed unhomogenized fats if the calves were already eating hay and grain.

Calves reared on filled milk are usually slow to start eating any feed other than milk. A few analyses of the feces showed a higher per cent of fatty acids and neutral fat in the feces of calves fed the unhomogenized oil. The haircoat of calves fed the unhomogenized oil appeared to have a normal oil content as judged by luster and handling qualities while the desquamated material from the denuded areas was always quite oily. Calves fed skim milk and either homogenized or unhomogenized oil grew at a rate below normal. Methods of at least patially overcoming this growth difficulty have been found and will be discussed in a later paper.

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THE EFFECT OF INCREASING THE NEGATIVE PRESSURE AND WIDENING OF THE VACUUM-RELEASE RATIO ON THE RATE OF REMOVAL OF MILK FROM THE UDDER[1, 2]

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According to Brodell and Cooper (1), six million hours per day or about 10 per cent of the total labor necessary for all farm operations are required to milk the cows in the United States. Dahlberg (3), Zehner (8), and Carter (2) have reported significant decreases in the time required for mechanical milking by the adoption of certain management procedures. Matthews, Shaw and Weaver (5), Foot (4), Matthews, Swett and Graves (6) and others have studied the rates of milking by machine, but no work has been reported on the effect of varying negative pressures and changes in pulsation ratios upon the speed of milk withdrawal.

EXPERIMENTAL METHODS

The relation of varying negative pressures and pulsation ratios to the rate of milk withdrawal was studied under three different plans. The first was to ascertain the amount of milk withdrawn in one minute at the height of milking by the use of a special apparatus. Two vacuum flasks were joined to the milking machine pail by a vacuum hose so that both had the same negative pressure. A standard teat cup was attached so that by the use of a Y-tube and forcep clamps the milk could be deflected into either flask. In the trials, milk was run into one flask until there was a full flow, then for exactly one minute the milk was shunted into the other flask and measurement made for the rate of flow per minute. Each of the four quarters was tested at 10.0, 12.5 and 15 inches Hg negative pressure and pulsation ratios of 1 : 1 and 3 : 1. The latter represents the relative time of vacuum application and release.

In the second plan the rates of milk withdrawal were ascertained for the entire udder with a standard milking machine for the first three minutes of milking. The milking pail was suspended from a scale and readings taken every 10 seconds. The rates for 1: 1 and 3: 1 pulsation ratios at 10, 12 and 14 inches Hg negative pressure were determined on three cows.

In the third plan, eight cows were milked by the same procedure followed in the second, except scale readings were taken to the "end point" of the milking. This point was arbitrarily established as the time when

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[2] The data in this paper are taken from a thesis presented by Vearl R. Smith in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota.

three successive scale readings were 0.3 pound or less or when two successive readings were identical, whichever occurred first. Also the third plan differed from the second in that a 2:1 pulsation ratio was compared with 1:1. The 3:1 and 2:1 pulsation ratios used in these experiments were obtained in specially constructed pulsators in which the vacuum was on for three and two times as long respectively, as the release.

In all experiments the cows were stimulated to let down their milk by 15- to 20-second wash and massage of the teats and udder two minutes before the machine was attached. The machine was operated at 50 pulsations per minute. In all cases the cows were stripped by manipulation of the teat cup assembly and the total milk ascertained for each milking. Except for plan one, in which a specially constructed machine was used, a standard double action, floor type milking machine was used throughout these experiments.

Under the first two plans, six, and in the third plan, ten, observations were made on each pulsation ratio and negative pressure level combination. In order to avoid differences in milk production levels due to a lapse of time, each pulsation ratio and negative pressure level combination was observed for one day each in series and the series repeated 6, 6, and 10 times, respectively, for the three plans.

RESULTS

The results obtained in the study of the rates of milk withdrawal for one minute for each of the three levels of negative pressure are presented for each of the quarters in table 1. It is to be noted that for any level of negative pressure, there is significant variation in the rate of milk withdrawal for the different quarters. For each of the quarters there is a significant increase in the rate of milking for each of the increases in negative pressures.

TABLE 1

Mean milk flow for one minute from individual quarters at three levels of negative pressure

Expt. No.	Negative pressure	Quarters			
		R.F.	R.R.	L.R.	L.F.
	Inches Hg	*ml.*	*ml.*	*ml.*	*ml.*
I	10.0	245	265	395	250
II	12.5	380	388	483	438
III	15.0	518	548	635	518
Per cent increase					
II over I		55.1	46.4	22.3	75.2
III over II		36.3	41.2	31.5	18.3
III over I		111.4	106.8	60.8	107.2

A much greater increase in milk flow is observed for the left front than for the left rear quarter, by increasing the negative pressure from 10.0 to 12.5 inches Hg, the increase being 188 and 88 ml., respectively. A smaller increase in milk flow from the left rear than the left front resulted when the negative pressure was increased from 12.5 to 15 in. Hg. The values were 80 ml. and 152 ml., respectively.

The percentage increase in milk withdrawal rates for each increment in negative pressures are also given in table 1. For three quarters the rates were more than doubled by increasing the negative pressure from 10 to 15 inches Hg, while for one, the left rear quarter, the increase amounted to only 60.8 per cent.

TABLE 2

Mean rates of milk withdrawal in pounds per ten seconds for 3 cows with 1: 1 and 3: 1 pulsator ratios at 10, 12, and 14 inches Hg negative pressures. Observations were made over three minute periods

	Inches Hg negative pressure						Average amount milk per milking
	10		12		14		
	Pulsator ratios						
	1:1	3:1	1:1	3:1	1:1	3:1	
							lbs.
Cow E390	0.65	0.74	0.72	0.85	0.80	0.89	16.8
E401	0.50	0.61	0.64	0.78	0.71	0.82	17.3
E405	0.52	0.58	0.64	0.69	0.74	0.84	17.7
Average	0.56	0.64	0.67	0.77	0.73	0.85	
	Ave. per cent increase from 1 : 1 ratio and 10 in. Hg						
		14.2	19.6	37.5	30.4	51.8	
	Ave. per cent of total milk in 3 minutes						
	56.9	68.7	69.3	80.7	78.0	87.4	

In table 2 is presented the summary of the results for three cows on which observations were made for a three-minute period with 1:1 and 3:1 pulsation ratios at 10, 12 and 14 inches of Hg negative pressure. Observations on 16 inches Hg negative pressure were discontinued because of the pain to the cow and congesting effect this level had on the teats with the 3:1 pulsation ratios. These effects were also quite noticeable for the 14-inch Hg level, and progressively less for the 12 and 10 inches Hg negative pressure, respectively.

It is to be noted that the rate of milk withdrawal increased progressively with each increment in the negative pressure used for both the 1:1 and 3:1 pulsation ratios. The 3:1 ratio pulsations milked faster than the 1:1 ratio by 14.2, 14.9 and 16.4 per cent, respectively, for 10, 12 and 14 inches negative pressures. The increases for the rates of milk withdrawal in per cent of milk obtained with the 1:1 at 10 inches of Hg negative pressure were

TABLE 3

Mean rates of milk withdrawal in pounds per 10 seconds up to "end point" for 8 cows with 1 : 1 and 2 : 1 pulsator ratios at 10, 12, 14 and 16 inches Hg negative pressures

	Inches Hg negative pressure								Ave. amt. milk per milking
	10		12		14		16		
	Pulsator ratios								
	1 : 1	2 : 1	1 : 1	2 : 1	1 : 1	2 : 1	1 : 1	2 : 1	
									lbs.
Cow A12	0.44	0.51	0.52	0.59	0.62	0.63	0.69	0.71	17.6
A30	0.51	0.58	0.64	0.72	0.72	0.84	0.74	0.89	16.3
A55	0.42	0.44	0.48	0.51	0.53	0.59	0.65	0.63	15.0
E401	0.42	0.46	0.55	0.59	0.62	0.70	0.71	0.77	13.0
E405	0.42	0.49	0.55	0.60	0.62	0.70	0.65	0.69	12.4
E408	0.49	0.56	0.61	0.62	0.65	0.70	0.69	0.70	13.0
E413	0.51	0.54	0.58	0.58	0.66	0.71	0.74	0.72	14.8
305	0.56	0.65	0.65	0.73	0.71	0.79	0.77	0.79	12.2
Average	0.47	0.54	0.57	0.62	0.64	0.71	0.71	0.74	
Ave. % increase from 1 : 1 ratio 10 ins. Hg		14.9	21.3	31.9	36.1	51.1	51.1	57.4	

19.6 and 30.4 for the 1 : 1 ratio pulsator, and 37.5 and 51.8 for the 3 : 1 ratio pulsator, respectively, for 12 and 14 inches Hg negative pressure. The rate of milking by the 3 : 1 ratio pulsation corresponded, roughly, to the rate obtained with the 1 : 1 ratio pulsation at a negative pressure 2 inches of Hg higher.

The mean rates of milk withdrawal up to the "end point" for 1 : 1 and 2 : 1 pulsation ratios at 10, 12, 14, and 16 inches Hg negative pressure levels are presented in table 3. These values are lower than in the previous experiment because they include a tapering off in milk flow rate as the "end point" is reached, as is shown in figure 1. The values, however, are more nearly what would be expected in practice, as stripping should begin at the "end point."

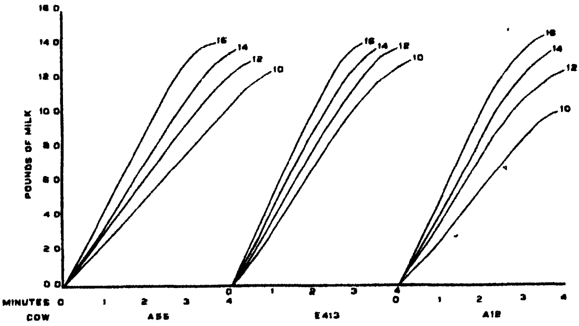

FIG. 1. Graphic presentation of rates of milking to the "end point" at 10, 12, 14 and 16 inches Hg negative pressures for 3 cows.

As in the previous experiment, increases in rates are observed for each increment in the negative pressure level. The 2:1 ratio pulsation withdraws milk more rapidly than the 1:1 ratio pulsation. The rate of milking by the former corresponds roughly to 2 inches higher negative pressure in the 2:1 ratio pulsation. Considerable variation is to be noted in the rate of increased milk flow by different cows at different negative pressure levels.

The time required in minutes and seconds to reach the "end point" with the two pulsation ratios and various negative pressure levels is presented in table 4 for the eight cows studied. There is considerable individual variation in the shortening of time at which the "end point" is reached as affected by the wider pulsation ratio and increments in the negative pressure levels. On the average, each two inches Hg increase in the negative pressure, over the ranges studied, caused a shortening of this period by 25 seconds. For any negative pressure level the 2:1 pulsation ratio, on an average, reduced the time to reach the end point approximately 15 seconds as compared with the 1:1 pulsation ratio. The 2:1 pulsator ratio with 16 inches Hg negative pressure averaged 2 minutes, 36 seconds to reach the "end point," while 4 minutes, 10 seconds was required by the 1:1 pulsation at 10 inches Hg negative pressure, or a difference of one minute 34 seconds.

TABLE 4

The time in minutes and seconds when the "end point" was reached in milking 8 cows with 1:1 and 2:1 pulsator ratios at 10, 12, 14 and 16 inches Hg negative pressure

	Inches Hg negative pressure							
	10		12		14		16	
	Pulsator ratios							
	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1
Cow A12	3:50	3:40	4:00	3:50	3:40	3:40	3:30	3:20
A30	5:00	4:30	4:10	3:40	3:30	3:10	3:10	2:50
A55	5:00	4:40	4:30	4:10	4:10	3:50	3:40	3:40
E401	4:20	4:20	3:50	3:30	3:20	3:00	2:50	2:30
E405	4:10	3:50	3:40	3:10	3:10	2:50	2:40	2:40
E408	3:40	3:20	3:20	3:10	3:00	2:50	2:50	2:40
E413	4:20	4:10	4:00	3:50	3:30	3:10	3:10	3:10
805	3:00	2:40	2:20	2:00	2:00	1:50	1:50	1:40
Average	4:10	3:54	3:44	3:25	3:18	3:03	2:55	2:36

In table 5 is presented a summary of the per cent of the total milk that was obtained when the "end point" was reached over the range of these studies. Again there is considerable individual variation. On the average, there is a progressive and significant increase in the per cent of the total milk obtained by the 2:1 pulsation ratio at 10 inches Hg negative pressure level and for both pulsation ratios with each increment in negative pressure level up to and including 14 inches Hg. On the average, a smaller percentage of the total milk was obtained for both pulsation ratios at the 16-inch Hg level of negative pressure. At this level an equal or larger proportion of the milk was obtained for five cows by the 1 : 1 pulsation ratio, and less for the 2 : 1 pulsation ratio for all but one cow. In one case (805) there was a progressive decrease in the completeness of milking at the "end point" for each increment in the negative pressure.

TABLE 5

The per cent of the total milking obtained when the "end point" was reached in milking 8 cows with 1 : 1 and 2 : 1 pulsator ratios at 10, 12, 14 and 16 inches Hg negative pressures

	Inches Hg negative pressure							
	10		12		14		16	
	Pulsator ratios							
	1 : 1	2 : 1	1 : 1	2 : 1	1 : 1	2 : 1	1 : 1	2 : 1
Cow A12	57.8	65.0	72.2	77.8	76.3	80.2	72.2	79.9
A30	94.2	94.3	96.7	96.4	94.2	96.7	96.2	94.2
A55	82.6	82.4	87.5	87.5	91.4	90.6	93.5	91.8
E401	86.5	91.7	93.7	93.8	95.1	95.7	95.1	94.4
E405	87.8	91.5	92.5	92.2	91.9	93.8	89.7	91.9
E408	88.4	88.6	92.4	92.9	89.2	91.6	90.8	90.1
E413 ...	88.9	93.1	92.9	92.4	91.7	92.6	93.5	92.4
805 .	81.8	83.0	78.3	77.4	70.9	70.2	65.8	63.7
Average ..	82.8	85.7	88.1	88.8	88.6	89.0	87.8	87.3

DISCUSSION OF RESULTS

While withdrawing milk for exactly one minute at the height of milking is an acceptable method to determine the effect of changes in the factors studied upon the maximum rates of milk withdrawal, it does not permit of an overall evaluation in practice. As subsequent experiments have shown, the completeness of milking is also affected by the factors studied. After preliminary trials the "end point" as herein used was chosen because at that point stripping should begin. The "end point" does not necessarily represent the extent to which the udder has been evacuated but rather a decline in the rate of flow of milk to a point where possible injury may result from the entrance of the negative force into the teat sinus.

In the cases where considerable milk was left in the glands when the "end point" was reached the decline in rate of milk withdrawal was caused by the upward crawling of the teat cups to partly or completely occlude one or more of the orifices between the teat and gland sinuses. Restoration of the maximum rate of milk withdrawal when the teat cups were pulled downward is proof of that fact. The tapering off in the rate of milk withdrawal toward the approach of the "end point," as shown in figure 1, may, in addition to the upward crawling of the teat cups, be due in part to a number of factors. Lowering of the intraglandular pressure due to the withdrawal of milk no doubt is an important contributing factor. Obstructions in the passageways for the milk and of course the more complete milking out of quarters producing less milk must also be considered.

While every increase in negative pressure resulted in an increased rate of milk withdrawal the rate of increase varied for different levels and for different cows. These variations can be explained on the basis that the negative force affects speed of milk removal in two ways. One is the effect upon the opening of the teat meatus, and the other is upon the rate of flow through the meatus. With every increase in negative pressure there is a proportional increase in the rate of flow through the meatus. To test out this point, skim milk was drawn through a hypodermic needle inserted through a rubber stopper at 10, 12, 14 and 16 inches negative pressure and rates of flow determined over a five-minute period. When the results for 14 trials at each level of negative pressure were plotted, a straight line increase with increased negative forces was observed.

The difference in the opening of the meatus therefore can account for great variation in the rates of increase in milk flow with increased negative pressure, both within and between cows. It seems obvious that the amount of force to open the meatus, maximally, will depend upon the tension of the sphincter muscles surrounding it. Other factors, such as stenosis of the passageways for the milk, either in the teat or gland sinuses or in the ducts of the gland, are often contributing causes for restriction of milk flow. Fibrotic growths, often present in the teat sinus, contribute greatly to an early occlusion of the passageway for milk in the milking process.

The notable shortening of the time to reach the "end point" with each increment of negative force is not the only criterion in speed of milking. In several cases a part of the time saved by reaching an earlier "end point" is offset by more time being required for removing larger amounts of milk as strippings. Why there should be, as an average, more complete milking at the "end point" with each increment of negative pressure up to and including 14 inches Hg is difficult to explain. The less complete milking with the higher negative pressure can be explained by a tendency for the teat cups to crawl upward earlier to partially or completely obstruct the passageways for the milk. The shape of the udder is a factor in the completeness of milking when the "end point" is reached. Cow 805 had funnel-shaped quarters, which offer less resistance to the crawling tendency than teats on udders with level floors. In her case, not only was the milking less complete at the low negative pressure level but progressively more milk was left in the udder when the "end point" was reached with each increment of the negative force.

As milk flows only when the inflation is distended, which in the normal pulsator is approximately one-half of the pulsation cycle, it would be expected that when the "vacuum" to release ratio is widened that milking rates would be increased. With both the 3:1 and 2:1 pulsation ratios rates of milking were increased but not proportional to the increased time over which the inflation is distended. Theoretically the 3:1 pulsation ratio should increase the rate of milk withdrawal 50 per cent and the 2:1 ratio 33.3 per cent over the 1:1 pulsation ratios. The observed increases were 17.6, 16.0 and 8.8 per cent respectively for 10-, 12-, and 14-inch Hg levels for the 3:1 ratio and 12.2, 7.9, 10.5 and 4.6 per cent respectively for 10-, 12-, 14-, and 16-inch Hg levels for the 2:1 ratio pulsations. The reasons for this discrepancy between the observed and the theoretical values are obscure. The tendency for a lesser increase in rates of withdrawal for wider pulsation ratios at the higher negative pressure levels can be explained by postulating that the level of maximum drainage rates from the gland is being reached.

While faster milking is desirable and from these studies can be accomplished by either widening the pulsation ratios or increasing the negative pressures or a combination of both there is obviously a practical limit beyond which one should not go. It was not the purpose of these studies to ascertain such a limit but attention must be directed to the fact that as the milking force is increased a greater traumatizing action is exerted upon the inside of the teat after milk ceases to flow out of the meatus as has been shown by one of us (7). Widening the pulsation ratios also has a tendency to increase the congestion in the teat. This latter condition could no doubt be largely overcome by increasing the pulsation rate so as to reduce the time the vacuum is applied to the teat with each pulsation.

SUMMARY AND CONCLUSIONS

1. Data involving observations on 12 cows are presented to show the effects of increasing the negative pressures and widening the pulsation ratios upon the rates of milk flow and the completeness of milking.

2. Rates of milk withdrawal at the height of milking were established for 1 minute at 10, 12.5 and 15 inches Hg negative pressures and rates per 10 seconds for the 1:1 and 3:1 pulsation ratios at 10, 12 and 14 inches Hg and for the 1:1 and 2:1 pulsation ratios at 10, 12, 14 and 16 inches Hg negative pressure levels.

3. Each increment in negative pressure in the range of 10 to 16 inches Hg, increased the rate of milk withdrawal but not at uniform rates for all cows.

4. Widening the pulsation ratio increased the rate of milk withdrawal but not in proportion to the increased time of vacuum application.

5. In general there was lesser increase in the rate of milk withdrawal by the wider pulsation ratios as the negative pressures increased.

6. The effect of negative pressure and widening of the pulsation ratios upon the completeness of milking was variable. In the lower ranges increased negative pressure affected a more complete average evacuation of the gland at the "end point." At 16 inches Hg negative pressure the reverse maintained.

7. Shape of the udder contributes to completeness of milking. Funnel-shaped quarters milk out less completely than those where the udder floor is level.

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THE KEEPING QUALITY OF SAMPLES OF COMMERCIALLY DRIED MILK PACKED IN AIR AND IN INERT GAS*

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The primary cause for the spoilage of dried milk is the autoxidation of the fat. The rate of autoxidation varies with the quality of the milk; the moisture, copper, and iron content; the method of processing; the temperature of storage; and the oxygen content of the atmosphere of the container.

With the advent of the war a special need arose for dried milk that would remain in good condition for at least six months to one year under severe conditions of handling and storage. At that time some of the dried milks were packaged in air and some in inert gas or gases.

Among the questions which confronted the potential users of large quantities of the product as well as manufacturers, were (a) what is the general keeping quality of commercial dried milk at different temperatures of storage; (b) how can the keeping quality of dried milks be predicted; and (c) to what extent can the keeping quality be augmented by packing the product in an inert gas? The work described herein was designed to answer these questions.

EXPERIMENTAL

Six lots of dried milk, approximately 1500 1-pound samples, were obtained from various manufacturers. One-half of each lot of samples had been packaged in inert gas or gases according to the practice of the manufacturer. The other half of the samples had been packaged in air.

Air- and gas-packed samples were stored at each of the following temperatures: 20°, 30°, 37°, 45°, and 55° C. (68°, 86°, 98.6°, 113°, 131° F., respectively). At regular time-intervals a container was removed from each set of samples stored at each of the temperatures, the contents were judged for off-flavors and odors, and the peroxide value was determined on the fat of each sample.

The determination of the oxygen content of the gas-packed samples was not made immediately but was made at a later date on several samples of each lot.

The samples were reconstituted and stored for off-flavors and odors by three judges. The peroxide value was determined as follows:

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The fat was extracted from 240 g. of dried milk with 560 ml. of hot carbon tetrachloride by agitating the mixture vigorously with a motor-driven stirrer for 10 minutes (Fig. 1). The mixture was poured on a folded filter and the residue washed four times with 15 ml. of hot carbon tetrachloride. The combined filtrate and washing were evaporated in an all-glass apparatus under vacuum until all the solvent had been removed.

FIG. 1. High-speed agitator with irregular shaped container.

Approximately 1 gram of the extracted fat was weighed accurately into a glass vial. The vial and contents were transferred to a 300-ml. Erlenmeyer flask and the sides of the flask were washed down with 25 ml. of a mixture composed of two parts glacial acetic acid and one part chloroform by volume. One milliliter of a saturated aqueous solution of KI was added and the mixture was rotated gently and placed in the dark for exactly 3 minutes. The contents of the flask were then diluted with 100 ml. of distilled water and 5 g. of sodium acetate was added. The flask was rotated to dissolve the sodium acetate and the liberated iodine was titrated with 0.002 normal sodium thiosulfate, a starch solution being used as an indicator. The number of milliliters of thiosulfate required to titrate the iodine liberated by 1 gram of fat is the peroxide value.

AIR-PACKED SAMPLES

Peroxide values. At relatively low storage temperatures the rate of formation of peroxides in milk fat is slow but exceeds greatly the rate of their destruction. As a result, the amount of peroxides usually increases logarithmically with respect to time. Any deviation from such a progressive rate of increase usually indicates an increase in the rate of decomposition of peroxides. This breakdown of peroxides occurs readily at the higher temperatures, but occurs also at relatively low temperatures if the time period of storage is relatively long, and results in tallowy off-flavors and odors.

Since the periods of storage in this investigation were usually relatively long, it was expected that excessive decomposition of peroxides might occur at the higher storage temperatures and would therefore make it impossible to follow accurately the rate of oxidation by means of the increase in the peroxide value.

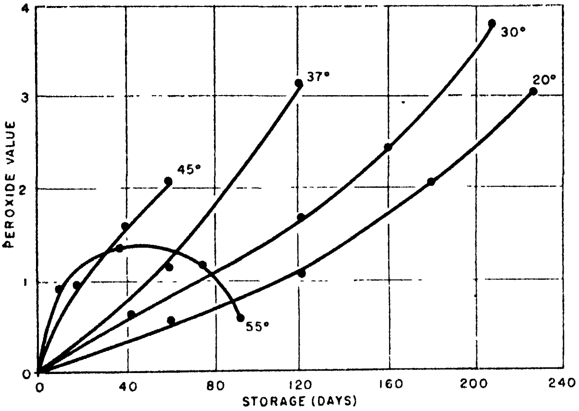

FIG. 2. The increase of peroxide value in the fat of a dried milk held at different temperatures.

The rate of peroxide formation in the fat in a representative sample of dried milk stored at the various temperatures is shown in figure 2.

The rates of peroxide formation at 20°, 30°, and 37° C. follow logarithmic courses and there is no indication of any considerable decomposition of the peroxide formed. However, at temperatuers of 45° and 55° the rates of autoxidation as determined by the peroxides present are not logarithmic, and indicate a progressive increase in the rate of decomposition of peroxides as the storage temperature increases.

Figure 3 shows the rates of accumulation of peroxides in the samples of the six lots of dried milk used, at storage temperatures of 20°, 30°, 37°, and 45° C. The relative keeping quality is indicated by the rates of peroxide formation.

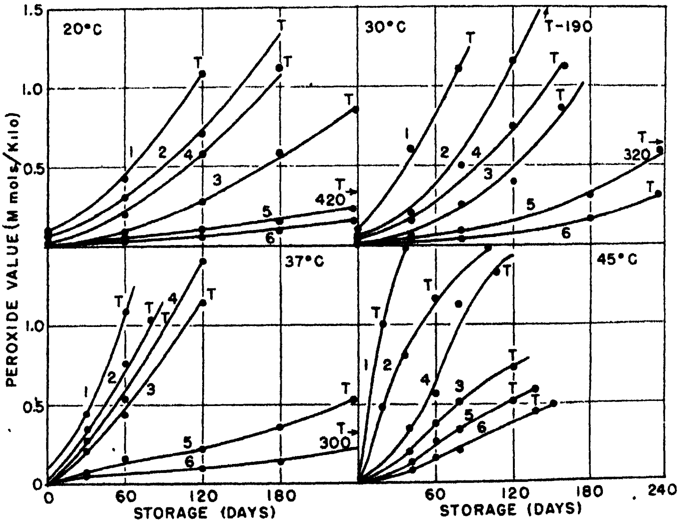

FIG. 3. The increase of the peroxide value in the fat of six dried milks held at different temperatures.

Although the peroxide values obtained were subject to some variations because of the small amounts to be determined, the values may be used to obtain the rate of autoxidation at different temperatures, provided, however, that the peroxide value chosen as an end point is not too great. The peroxides are more stable at lower than at higher concentrations. Figure 4 shows the time in days required for the fat in the dried milks Nos. 1, 2, 3, and 4 to attain a peroxide value of 1.00, plotted logarithmically against the tempera-

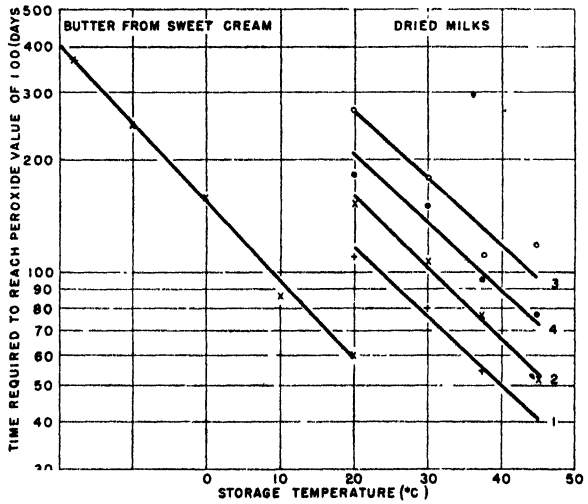

FIG. 4. The time required for the development of a peroxide value of 1.0 in dried milks and sweet cream butter.

tures of storage. Values for sweet-cream butter (1) stored at −17° to 20° C. are also given in figure 4.

The values for dried milks Nos. 5 and 6 were not included, since in neither of these did the peroxide value of the fat reach a value of 1.00 before they were judged tallowy. Although some of the values do not conform well to the curves, the differences in the rates of oxidations as related to the differences in the storage temperatures seem to be in each case approximately the same as for those of sweet-cream butter. For each decrease of 10° C. in the storage temperature, the number of days of storage required to effect the same degree of autoxidation increases approximately 1.6 times.

Off-flavors and odors. The rate of peroxide formation was of value in determining the relative rates of autoxidation of fats and can be used as an index of the comparative keeping qualities of dried milks packed in air, if the storage temperature is not above 45° C. However, the amount of peroxides formed may not be the same in all cases in which sufficient amounts of peroxides have been decomposed to produce tallowy flavors. This is illustrated in figure 3, in which the time when the milks were first judged tallowy (T) is indicated on the curves for the rate of peroxide formation.

TABLE 1

Comparison of the keeping quality of six lots of dried milk, based on the flavor and on the peroxide value at the end of the second sampling period

Time of storage	Temperature	Increasing off flavor →						Increasing peroxide value →					
days	*°C.*	*lot number*						*lot number*					
30	55	6	5	4	3	2	1	6	5	3	4	2	1
40	45	6	5	4	2	3	1	6	5	3	4	2	1
60	37	6	5	4	3	2	1	6	5	3	4	2	1
80	30	6	5	4	2	3	1	6	5	3	4	2	1
120	20	6	5	4	3	2	1	6	5	3	4	2	1

In general, tallowiness occurs at a lower peroxide value when the periods of storage are long. There is also some evidence in these results that peroxides decompose more readily in some dried milks than in others. This fact may lead to inconsistencies in the comparative results obtained by the organoleptic test and the peroxide value methods of judging keeping quality. The rates of peroxide formation in the fat can be used as a fairly reliable test of the relative keeping quality of dried milks. This is shown also in table 1 in which is given the order of increasing off-flavor and peroxide value of six lots of dried milk at the second sampling period.

INERT GAS PACKING

As indicated previously, one-half of each lot of samples had been packed in inert gas by the manufacturers. These samples were stored at the same temperatures as the air-packed samples and their peroxide values determined and flavors and odors judged at the same intervals.

Of the gas-packed samples four lots had oxygen concentrations of approximately 3 to 4 per cent, the percentage differing somewhat between containers.

Peroxide formation. The rate of formation of peroxides in these samples was low and in most cases did not reach a value which could be considered significant. In many cases the peroxide value reached a maximum of approximately 0.50 and remained at this value for a long time. Often samples were judged tallowy when there had been little change in the peroxide value over a long period of storage. This can be accounted for only by the assumption that the rates of formation and destruction of peroxides were almost equal and that eventually enough oxygen was absorbed and its initial reaction product destroyed to render the product tallowy.

TABLE 2

The time of storage at 37° C. necessary to produce a tallowy odor and flavor in dried milk samples packed in air and inert gas

Sample	O_2 content	Time of storage
	%	*days*
1. Air		60
Inert gas	3.86	150
2. Air		60
Inert gas	4.69	210
3. Air	...	120
Inert gas	3.38	240
4. Air		120
Inert gas	3.94	270

It is evident therefore that the rate of peroxide formation in the fat of the gas-packed samples cannot be used as a measure of the relative keeping quality of the dried milks.

The number of days of storage at 37° C. required to develop the first definitely tallowy flavor in the air- and gas-packed samples of each of the four lots is given in table 2.

These values indicate that the keeping quality of the dried milks packed in inert gas of 3 to 4 per cent oxygen concentration was from two to three times that of samples of the same product packed in air.

Although the oxygen concentration of the gas in the containers of each lot was not the same, the results indicate that dried milks of relatively low keeping quality are benefited to a greater extent by packing in gas than are those of a better keeping quality.

The other two lots of gas-packed samples (Nos. 5 and 6) differed greatly in oxygen content. Consistent data were not obtained upon their keeping quality.

DISCUSSION AND CONCLUSION

The samples used in this work were obtained and studied in 1942. The results indicate clearly that there was a great variation at that time in the keeping quality of commercially prepared dried milks. In most cases they were of such quality that those packed in air could not be relied upon consistently to withstand severe conditions of storage for 6 months to 1 year without developing oxidized flavors and odors.[1] However, those packed in an inert gas of an oxygen content of approximately 3 to 4 per cent had a much better keeping quality than those packed in air.

The tests of samples packed in air and stored at 20°, 30°, 37°, 45°, and 55° C. indicate that the rate of peroxide development at the temperatures up to 45° can be relied upon as a fairly accurate measure of the rate of autoxidation and as a measure of the relative keeping quality of dried milks, provided the criterion used is a relatively low peroxide value. The rate of peroxide development was not, however, necessarily an index of the time of storage necessary to develop tallowy odors and flavors in a gas-packed sample.

In general those samples which had an initial peroxide value of zero were of superior keeping quality.

Slight off-flavors and odors could usually be detected when the peroxide value reached approximately 0.50. When the keeping quality is determined by using the first off-flavor in the organoleptic test and a low peroxide value as criteria, the two tests agree reasonably well. The two tests do not agree as well when the criterion in the organoleptic test is too pronounced a flavor or when the criterion in the peroxide value test is too high. Therefore the peroxide value may be used as an index of the probable storage quality.

By the use of relatively high storage temperatures the keeping quality test can be greatly accelerated, since the rate of oxidation of the fat in a milk of good quality increases by a factor of approximately 1.6 × for each increase of 10° C. in the storage temperature.

Temperatures of 37° and 45° C. seem to be satisfactory to use in accelerated storage tests. A temperature of 55° was found unsatisfactory because of the destruction of peroxides and the development of discoloration and off-flavors not connected with fat oxidation.

Although the peroxide value may be used to determine the relative keeping quality of dried milks packed in air, the keeping quality of dried milks packed in an inert gas can best be determined by organoleptic tests made during storage to determine the onset of off-flavor and odor.

REFERENCE

(1) Holm, G. E., Wright, P. A., White, Wm., and Deysher, E. F. Jour. Dairy Sci., 21: 385–398. 1938.

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[1] Great advances have been made in the manufacturing and packaging of dried milk in the past two years and the quality of the dried milks now manufactured is generally better than was the quality of some of those used in this work.

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The Constitution and By-laws of the American Dairy Science Association provides for the publication of the names of the nominating committee in the January issue of the Journal of Dairy Science. It is the duty of this Committee of which **G. M. Trout** is the present chairman to send its report to the secretary not later than April 1. The secretary will then send out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a member of the Association, it is your obligation to express your wishes for officers and directors to any member of this Committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president.

The Nominating Committee will give consideration to the wishes of the members as expressed by correspondence and also will consider additional candidates. They have been instructed to study the list of past officers and directors (see pages 803 and 804 of the August, 1943, Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members.

This change in the Constitution was made so that our Association could elect its officers in a democratic way. This can only be done, however, if our members are prompt in giving their opinions freely to the Nominating Committee.

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THE OCCURRENCE AND SURVIVAL OF BRUCELLA ABORTUS IN CHEDDAR AND LIMBURGER CHEESE*

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Most dairy products reach consumers after being subjected to a heat treatment sufficient to destroy any pathogenic bacteria that might be present, but there is still much unpasteurized Cheddar cheese made from raw milk. Since such cheese has not been found to cause undulant fever in man, one might reasonably expect that *Brucella abortus* dies or becomes non-infectious during normal cheese curing. It is known that the dairy cows of the United States are often infected and that infected cows often give milk containing these bacteria. Conditions in the early stages of the manufacture of Cheddar cheese favor bacterial development and the bacteria are concentrated in the fresh curd. Some of the cheese might be infected so the question of the survival of *Brucella abortus* in Cheddar cheese has public health significance.

This study was undertaken to show the length of time that *Brucella abortus* survives in Cheddar cheese. First a preliminary survey was made on cheese milk in New York State. Then a study was conducted on cheese made from milk inoculated with cultures of the organism recently isolated from naturally infected milk. Tests were made on cheese manufactured from milk from selected reacting cows that were found by test to be producing milk heavily infected with *Brucella abortus*. Finally, a survey was conducted showing the contamination naturally occurring in milk of cheese-producing areas in New York and Wisconsin, where brucellosis had not been eradicated from the dairy herds and the survival of *Brucella abortus* in naturally infected commercial Cheddar cheese was determined.

LITERATURE

The literature contains comparatively little information on the viability of *Brucella abortus* in cheese. There is no evidence that undulant fever has

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* This investigation was aided by a grant from the National Cheese Institute and was originally proposed by its research committee.

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been contracted as a result of eating cheese made solely from cow's milk. On the other hand, cheese made from infected, unpasteurized goat's milk has been found to be highly infective for man, especially when consumed soon after manufacture. As with most bacteria, the survival of Brucella in milk and milk products is influenced greatly by such factors as duration of storage, temperature, and acidity.

Eyre (6) isolated *Brucella melitensis* from goat's cheese up to 48 hours after manufacture. After this time, he states that recovery was impossible due to the increased growth of lactic acid bacteria. Versilova (16) found that *Brucella melitensis* remained viable and infective for guinea pigs for 45 days at 11° to 14° C. in Brynza cheese prepared from milk inoculated with *Brucella melitensis* after pasteurization. In sheep's milk with a pH of 6.8 to 6.0 *Brucella melitensis* was viable 22 to 40 days at a temperature of 11° C. At pH 5.0 to 4.0 it remained viable 15 days, but never more than 30 days, whereas in milk kept at 37° C. it frequently died the first few days.

Peres and Granon-Fabre (10) report the recovery by guinea-pig inoculation of *Brucella melitensis* from cheese made from naturally infected goat's milk which had been maturing for 20 days, but not from the samples maturing 6, 10, or 17 days. Veloppe and Jaubert (15) report 14 cases of undulant fever due to fresh cheese. There were two deaths. They emphasize that it is common practice to mix the milk of goats, sheep, and cattle. The authors conclude that infected cheese is harmless after 30 days.

Carpenter and Boak (4) injected, into guinea pigs, 82 samples of cheese of various kinds, 72 of which were imported. Of the 72 imported samples, 30 were manufactured in Italy, 14 in France, 13 in Switzerland, 6 in Holland, and the remainder in England, Germany, and North and South America. The majority of the types examined were Swiss, Roquefort, Reggiano, Gorgonzola, and Edam cheeses. There was no indication as to the age of any of the foreign or domestic types. Infection with *Brucella abortus* or *Brucella melitensis* was not found in any of the guinea pigs injected with these samples.

Smith (11) examined samples of Cheshire, Cheddar (red and white), Double Gloucester, Bel Paese, Convalli, Camembert, Wenslet, Roquefort, Gouda, Stilton, Edam, Gorgonzola, Chilvern, Parmesan, and locally prepared Scotch cheese. Twenty-three of the cheeses were made in England, 6 were colonial in origin, 28 foreign, and 6 local. The samples were prepared by passing the cheese through a fine tissue mincer, thereafter adding saline to make a fine paste, and then injected in 5-ml. amounts into each of two guinea pigs. Examination of all 63 samples failed to show the presence of *Brucella abortus* or *Brucella melitensis.*

Stiles (12) examined 19 cheese samples, of which 8 were listed as positive for *Brucella melitensis* when injected into guinea pigs. Four samples were of the Feta type made from raw goat's milk, 3 of the Romano type made from raw goat's milk, and one was imported (Mexico) yellow cream cheese from cow's milk. It is quite possible that this cheese was made from a combination of goat's and cow's milk in view of the fact that it is common practice in some countries to combine any types of milk available. The reaction of the cheeses was given as pH 6.8. The "probable age" of the positive cheeses at the time of study was from 38 to 100 days. One cheese made from cow's and goat's milk was listed as positive after one year. However, the positive findings in this case are open to question inasmuch as *Brucella melitensis* was not recovered from the inoculated guinea pig. Lesions and cultures were negative. The blood titer of 1:200 in this case could have been caused by a large number of dead Brucella organisms present in the cheese. In a footnote, the author states that 6 additional samples of Colorado goat cheese of the Romano (Incannestrato) type had been examined since the preparation of the paper. Of this number, 4 were negative by guinea pig tests, and 2 proved positive. The 2 positive samples were from lots of cheese manufactured 64 and 70 days prior to the examination. The guinea pigs showed typical lesions, and yielded pure cultures of *Brucella melitensis.*

Lerche (8) states that the viability of *Brucella abortus* in cheese is greatly dependent on the acidity of the product. In all cheese, such as Landekäse, made by the natural souring of milk, the organism dies rapidly. However, in milk artificially infected with *Brucella abortus* and made into Weisskäse and Frühstückkäse by the rennet process, it persisted up to 24 days. Drescher and Hopfengartner (5) made up several types of cheeses from milk that had been heavily inoculated with *Brucella abortus.* At intervals, samples of cheese from near the rind and from the inner portion were taken for guinea-pig inoculations and cultures. One gram of cheese, plus 1 ml. of physiological saline solution were inoculated intraperitoneally in amounts of 0.5, 1, 2, and 3 ml. in each guinea pig. In Delikatkäse the organism was recovered from the rind and inner portion at 35, but not at 42 days. The cheese had been stored at 4° to 5° C. In Tilsiterkäse the organism lived up to 35 days as determined by culture and guinea-pig inoculation (49 days by inoculation only) in the rind, and 91 days by each method in the inner portion of the cheese. In Emmentalekäse *Brucella abortus* survived 49 days in both rind and inner portion. Lichtenstein (9) calls attention to the effect of acidity and temperature on the survival of *Brucella abortus* in milk and milk products. Thompson (14) quotes Voille to the effect that in cheese, especially Roquefort, *Brucella abortus* can remain viable for a period of two months. In view of the fact that Roquefort cheese is made from ewe's milk, it is probable that this organism was of the *melitensis* rather than the *abortus* variety. There is no indication as to whether the cheese was made from naturally infected milk, or from milk heavily inoculated with the organism, as is so frequently done in experimental work.

EXPERIMENTAL

In the summer of 1942 samples of milk were collected at random in the two principal cheese-producing areas of New York State to ascertain the prevalence of *Brucella abortus* in commercial cheese milk. The milk samples were taken from the cheese vats in the factories just prior to adding rennet. They were iced and delivered by car directly to the research laboratory for examination. Samples of cheese from each vat were marked for later identification in case the milk was positive so that the cheese could be tested later for Brucella. Both Cheddar and Limburger cheese factory milks were taken for the study. No consideration was given to the presence of brucellosis in the herds producing milk for the cheese factories. The data on Cheddar cheese are reported as experiment 1, on Limburger cheese as experiment 2.

Milk was artificially inoculated with *Brucella abortus* to give a heavily infected milk. A local dairy herd in which practically all cows reacted was found to be eliminating Brucella in sufficient numbers to give positive results with guinea pigs and a plate count of about 500 per ml. *Brucella abortus* was isolated from this milk and enough of the culture was added to the naturally infected milk to give a total count of approximately 1000 *Brucella abortus* per ml. This milk, in the early spring of 1942, was made into Cheddar cheese on three different days. Each batch of milk was tempered to 30° C., inoculated with 1 per cent of good lactic starter and the *Brucella abortus* culture. The first batch of milk (B.A. 1) was incubated for 30 minutes before adding rennet and the acidity developed slowly to 0.35 per cent in the whey at milling time even though the time from adding culture to milling was 6 hours. This is a low acid cheese. The second batch of milk (B.A. 11) was handled like the first but the acidity developed normally and was 0.56 per cent in the whey when the curd was milled. The B.A. 21 or third batch of milk was incubated with lactic starter and Brucella culture for one hour at 30° C. before rennet was added. The whey acidity at milling was 0.60 per cent. This cheese was well developed in acidity. The standard Cheddar cheese process was employed with a cooking temperature of 37.8° C. The cheeses were cured at 4.4° C. because a low temperature favors survival for long periods, as shown by Campbell and Gibbard (3) for *Eberthella typhosa,* and Yale and Marquardt (18) for *Streptococcus pyogenes.*

Tests were made on the original raw milk, the milk inoculated with *Brucella abortus,* the fresh curd as taken from the vat after salting, and the cheese when one week, two weeks, one month, three months, six months, and one year old. The data are reported as experiment 3.

Cheddar cheese was also manufactured in the laboratory from uninoculated milk that contained the heaviest natural contamination with *Brucella abortus* that was found by selecting milk from individual cows. In the fall of 1942 tests were made on the milk produced by about 30 reacting cows, and two cows were selected because they were giving large numbers of Brucella in their milk. The *Brucella abortus* plate counts were 700 to 800 per ml. Plate counts of Brucella in milk were made according to the method of Huddleson (7). The milk from the two selected cows was accumulated for two days and then made into cheese. This was repeated on three different days. The methods of manufacturing the cheese were the same as those previously employed. The milks for the three batches were designated as B.A. 31, B.A. 41, and B.A. 51. The raw milk, curd, and cheese were tested for viable Brucella as in the previous test. The data are reported as experiment 4.

Finally, in the early months of 1944 a survey was made of the prevalence of *Brucella abortus* in cheese milk and Cheddar cheese under commercial conditions in areas in Wisconsin and New York where brucellosis is prevalent. It is known that brucellosis has been eradicated from some areas and there is little probability of milk being contaminated with *Brucella abortus.* Milk from such areas was avoided. Factories were chosen whose patrons were known to have made little or no effort to test their cows for brucellosis. After the cheese vat contained the entire quantity of milk to be made into cheese, the milk was stirred and a sample placed into a bottle containing sufficient boric acid powder to make a 0.5 per cent solution in the milk. The boric acid inhibits the growth of bacteria and delays the souring of the milk, but does not kill *Brucella abortus* in this concentration. The milk sample was cooled. In the afternoon a sample of the salted cheese curd was placed in another bottle. Both samples were then sent to the laboratory by special delivery air mail or by automobile. One cheese from each batch was marked to be saved if wanted later for testing in case the milk proved positive for *Brucella abortus.* The cheese was held at the factory for a few days and was then stored in the commercial warehouse at the usual temperature of 1.1° to 2.8° C. At the end of five weeks when the results on the milk were available, the cheeses made from positive milks were secured for further analysis. All tests on the cheeses were made as soon as secured, but this time varied from 41 to 84 days. The cheeses were tested a second time after a lapse of 30 to 60 days. The plan was to test the cheeses when two and four months old. The data are reported as experiment 5.

When milk was received at the laboratory it was placed in the refrigerator overnight to permit creaming. In experiments 1, 2, and 5, four guinea pigs were each injected subcutaneously with 5 ml. of the gravity cream and after five weeks the guinea pigs were killed and examined for Brucella infection. In experiments 3 and 4, eight guinea pigs were used for each milk sample. The cheese was prepared by taking a total of 50 grams from at least three different places in the cheese for experiments 3 and 4, whereas 25 grams were used in experiment 5. The cheese was placed in a sterilized Waring Blendor with 100 ml. of sterile physiological saline solution and agitated for ten minutes to produce a smooth creamy mixture. The mixture was then filtered through one layer of cheesecloth and 2-ml. amounts were injected subcutaneously into each of the four or eight guinea pigs. Each guinea pig received the equivalent of about 0.8 gram of the laboratory-made cheese and about 0.42 gram of the commercial cheese. The smaller dose of the commercial cheese was the maximum amount that the guinea pigs could tolerate.

Positive tests were those in which the guinea pigs, five weeks after injection, gave positive blood agglutination reactions, characteristic lesions in the slaughtered animals, and recovery of *Brucella abortus* from the spleen. Inasmuch as dead *Brucella abortus* cause a positive blood agglutination test, no animal was regarded as positive unless the organism had been recovered from the spleen by bacteriological culture methods.

RESULTS

Experiment No. 1. Brucella abortus in Milk and Cheddar Cheese from Commercial Cheese Factories Selected at Random

Milk samples were collected from 15 different vats at 11 different commercial Cheddar cheese factories in New York State. Much to our surprise, all 15 milk samples failed to show the presence of *Brucella abortus* as determined by animal inoculation. The organisms were not viable, or had been so greatly diluted in the pooled milk that their presence could not be demonstrated by the method used. The cheeses were therefore released.

Experiment No. 2. Brucella abortus in Milk and Limburger Cheese from Commercial Cheese Factories Selected at Random

Ten milk samples were obtained from 10 different factories making Limburger cheese in New York State. The fresh curd (24 hours old), made from four of these vat samples, was also obtained and injected into guinea pigs. Eight of the 10 milk samples were negative for the presence of *Brucella abortus.* Two milks contained the organism in sufficient quantity to cause infection in the inoculated guinea pigs. The 24-hour-old curd made from these two positive milk samples failed to show the presence of the organism. Samples from these two cheeses were later injected into guinea pigs after storage for 57 days. *Brucella abortus* could not be demonstrated.

Experiment No. 3. Brucella abortus in Cheddar Cheese Manufactured from Milk from a Highly Infected Herd, and Artificially Inoculated with Brucella abortus

This experiment was initiated in an effort to determine the viability of *Brucella abortus* in Cheddar cheese made up under laboratory conditions from milk obtained from an infected herd in which some of the animals were known to be eliminating the organism in the milk. The milk, as determined by bacteriological count, contained approximately 500 viable *Brucella abortus* per ml. Just before the milk was manufactured into cheese, enough viable *Brucella abortus* culture was added to bring the total Brucella count to approximately 1,000 organisms per ml. The plan was to determine by guinea pig inoculation the presence of viable Brucella in fresh cheese curds, and in cheeses made from inoculated milks stored for one week, two weeks, one month, three months, six months, and one year, respectively.

In series B.A. 1–9 the organism was fully viable in the raw milk, fortified milk, fresh cheese curd, and the stored cheeses up to and including three months. At six months, all guinea pigs were positive but the lesions were slight, showing that they had received comparatively few viable organisms. At one year, all the guinea pigs were negative, as shown in table 1.

TABLE 1

Survival of Brucella abortus in Cheddar cheese manufactured from milk from a highly infected herd, and fortified with Brucella abortus (Exper. No. 3)

Product and age	First series		Second series		Third series	
	Sample	Results	Sample	Results	Sample	Results
Raw milk	B.A. 1	+	B.A. 11	+	B.A. 21	+
Raw milk plus *Brucella abortus*	B.A. 2	+	B.A. 12	+	B.A. 22	+
Fresh curd	B.A. 3	+	B.A. 13	+	B.A. 23	+
Cheese, 1 week	B.A. 4	+	B.A. 14	+	B.A. 24	+
Cheese, 2 weeks	B.A. 5	+	B.A. 15	+	B.A. 25	+
Cheese, 1 month	B.A. 6	+	B.A. 16	+	B.A. 26	+
Cheese, 3 months	B.A. 7	+	B.A. 17	+	B.A. 27	+
Cheese, 6 months	B.A. 8	+*	B.A. 18	+*	B.A. 28	+†
Cheese, 1 year	B.A. 9	–	B.A. 19	–	B.A. 29	–

* Guinea-pig lesions slight. Cultures positive.

† Five guinea pigs negative. Three showed slight lesions. Cultures positive.

In series B.A. 11–19 results were identical with those obtained with series B.A. 1–9.

In series B.A. 21–29 the organism was fully viable at three months (table 1). At six months, five guinea pigs were negative. The three others were positive, but showed slight lesions. No infection was present in any of the guinea pigs inoculated with the sample one year old.

Experiment No. 4. Survival of Brucella abortus in Cheddar Cheese Manufactured from Milk from Selected Cows in a Highly Infected Herd, That Were Found to Be Eliminating Brucella abortus in the Milk

The cheese for this experiment was made up and handled in the same manner as that used in experiment 3 except that the milk was from a different reacting herd, and the milk was not fortified by the additional *Brucella abortus*. The milk contained 700 to 800 viable *Brucella abortus* per ml.

Inasmuch as the milk was only from two animals found to be eliminating the organism in the milk in large numbers, there was no dilution of the organism as occurs in pooled samples of milk from a given herd or area. We may therefore assume that the concentration of organisms was considerably greater than would occur under commercial conditions.

In series B.A. 31–39 the organisms were viable in the raw milk, fresh curd, and the stored cheeses up to and including one month, as shown in table 2. At three months all eight guinea pigs were positive, but the lesions were slight. At six months, five guinea pigs were negative, and three positive. In the three positive animals the lesions were slight. All guinea pigs inoculated with the one-year-old sample were negative.

In series B.A. 41–49 all samples were strongly positive up to and including the one-month cheese sample (table 2). At three months, seven guinea pigs were negative; one showed slight lesions but gave a positive culture. All guinea pigs inoculated with the six-month and one-year samples were negative.

TABLE 2

Survival of Brucella abortus in Cheddar cheese manufactured from milk from two selected cows in a highly infected herd, that were found to be eliminating Brucella abortus in the milk (Exper. No. 4)

Product and age	First series		Second series		Third series	
	Sample	Results	Sample	Results	Sample	Results
Raw milk	B.A. 31	+	B.A. 41	+	B.A. 51	+
Fresh whey	B.A. 32	+	B.A. 42	+	B.A. 52	+
Fresh curd	B.A. 33	+	B.A. 43	+	B.A. 53	+
Cheese, 1 week	B.A. 34	+	B.A. 44	+	B.A. 54	+
Cheese, 2 weeks	B.A. 35	+	B.A. 45	+	B.A. 55	+
Cheese, 1 month	B.A. 36	+	B.A. 46	+	B.A. 56	+
Cheese, 3 months	B.A. 37	+*	B.A. 47	+ –‡	B.A. 57	+*
Cheese, 6 months	B.A. 38	+ –†	B.A. 48	–	B.A. 58	+*
Cheese, 1 year	B.A. 39	–	B.A. 49	–	B.A. 59	–

* Guinea-pig lesions slight. Cultures positive.

† Five pigs negative. Slight lesions in three. Cultures positive.

‡ Seven guinea pigs negative. Slight lesions in one. Cultures positive.

In series B.A. 51–59 all samples were strongly positive up to and including the one-month sample. At three months and six months, all guinea pigs were positive, but the lesions were slight. All guinea pigs injected with the one-year sample were negative, as shown in table 2.

Experiment No. 5. Brucella abortus in Milk and Cheddar Cheese from Commercial Cheese Factories in Areas Where Brucellosis Had Not Been Eradicated from Producing Herds

Commercial cheese-milk samples, and fresh cheese curd were obtained for test for the presence of *Brucella abortus* in areas where little or no attempt had been made to eradicate brucellosis from the herds. Milk and fresh curd were obtained from 22 different vats in 18 separate Wisconsin factories, and from 12 different vats in 9 New York factories. A smaller amount of cheese was inoculated into each guinea pig because of the fact that the pigs could not tolerate the larger amount used in the previous experiments. The probable explanation is that the milk used in experiments No. 3 and 4, which had bacterial counts below 10,000, was of a higher sanitary grade than that customarily used in commercial cheese manufacture.

All cheeses were held at the factory until the results of the inoculations for the presence of *Brucella abortus* were obtained. Cheeses made from positive milk were then purchased and brought to the laboratory, stored at 1.7° to 4.4° C., and examined further for the presence of *Brucella abortus*. It was our plan to obtain the cheeses promptly to test them at 60 days of age, but because of misunderstanding and food rationing, receipt was delayed in four instances.

Of the 22 lots of milk from the Wisconsin factories, five were positive for *Brucella abortus*. The remainder were negative as shown in table 3. Of the five positive samples, the cheeses could not be obtained in two instances. The cheeses had been released by mistake and could not be traced. The cheeses made from the other three samples were obtained and subjected to further testing for *Brucella abortus*. One cheese was negative for *Brucella abortus* on the first test at 66 days and later on second test at 144 days; the other two cheeses were negative at 84 and 115 days after manufacture. It is not known whether the cheeses were negative at earlier dates.

Of the 12 milk and fresh curd samples from the New York factories, eight milk samples were negative and four were positive (table 3). One sample of positive milk gave fresh cheese curd that was negative. All four samples of cheese from the positive milk were negative in first test. Two of these four were negative for *Brucella abortus* at 74 days and 104 days. The other two were found to be negative at 41 days and also at 65 days. Again, it is not known just how soon after manufacture these cheeses actually became negative as they were negative in first test.

DISCUSSION

A very serious obstacle in the study of the survival of most pathogenic bacteria in commercial cheese is the difficulty of obtaining vats of cheese milk known to be infected with the bacteria under observation which would normally be made into cheese. It is generally impossible to determine the extent of the contamination that naturally occurs in the milk or to follow the survival of the bacteria in the cheese made under commercial conditions as no one can predict in advance that a given vat of milk is infected with specific disease-producing bacteria. Under such circumstances it has been the accepted research practice to artificially fortify milk with selected patho-

TABLE 3

Brucella abortus in milk and Cheddar cheese from commercial cheese factories in areas where brucellosis had not been eradicated from the herds (Exper. No. 5)

No.	Factory	Milk	Curd	Cheese†	No.	Factory	Milk	Curd	Cheese†
1	Wis. G.V.	–	–		18	Wis. J.	–	–	
2	Wis. W. 1	–	–		19	Wis. Sp.	–	–	
3	Wis. W. 2	–	–		20	Wis. Do.	–	–	
4	Wis. B.A.	+*	+*	Negative at 66 days Negative at 144 days	21	Wis. G.A.	+*	+*	Unobtainable
5	Wis. B.B.	–	–		22	Wis. S.B.	+*	+*	Unobtainable
6	Wis. Bo. A.	–	–		23	N.Y. C. 1	–	–	
7	Wis. Bo. B.	–	–		24	N.Y. C. 2	+	+	Negative at 74 days Negative at 104 days
8	Wis. H.	–	–		25	N.Y. C. 3	–	–	
9	Wis. C.	–	–		26	N.Y. C. 4	+	+	Negative at 74 days Negative at 104 days
10	Wis. D.A.	–	–		27	N.Y. L. 1	–	–	
11	Wis. D.B.	–	–		28	N.Y. L. 2	+	+	Negative at 41 days Negative at 65 days
12	Wis. S.	–	–		29	N.Y. L. 3	–	–	
13	Wis. H.C.	–	–		30	N.Y. L. 4	+*	–	Negative at 41 days Negative at 65 days
14	Wis. Na.	–	–		31	N.Y. L. 5	–	–	
15	Wis. G.M.	+*	+*	Negative at 84 days Negative at 115 days	32	N.Y. L. 6	–	–	
16	Wis. O.	+*	+*	Negative at 84 days Negative at 115 days	33	N.Y. L. 7	–	–	
17	Wis. C.L.	–	–		34	N.Y. L. 8	–	–	

* Guinea pigs showed slight lesions. Cultures positive.

† The only tests made on the cheeses are those reported in the table.

gens and then manufacture this milk into cheese under laboratory conditions. All of the controlled experiments on the survival of *E. typhosa, Mycobacterium tuberculosis,* and *S. pyogenes* in cheese have been conducted in this manner. Such experiments yield valuable information but may not apply to commercial cheese as the size of the inoculation naturally occurring in milk is unknown and the actual longevity and virulence of the bacteria causing the contamination may differ from those cultured in the laboratory. The only other alternative is to study cheese that has been suspected of producing a cheese-borne epidemic to determine the probable age of the cheese and to attempt to isolate the bacteria involved.

Fortunately for research purposes, the survival of *Brucella abortus* in cheese may be studied more advantageously than other pathogens. Freshly isolated cultures were used to fortify milk for cheese making, but it was also possible to find naturally infected milk delivered to factories in areas where brucellosis had not been eradicated. Milks from many sections were tested to obtain supplies containing *Brucella abortus* which provided naturally infected milk that could be made into cheese. Hence, the survival of *Brucella abortus* could be studied in cheese made by accepted laboratory procedures and under practical conditions.

The data on Cheddar cheese manufactured in the laboratory from milk fortified with a culture of *Brucella abortus* or from milk produced by reacting cows whose milk was found to be highly contaminated with these bacteria showed that *Brucella abortus* survived in cheese at 4.4° C. for six months but not for one year. These bacteria tended to die a little more rapidly in cheese made from the milk selected as being naturally heavily contaminated. These data agree well with those recently published by Campbell and Gibbard (3) for *E. typhosa* in Cheddar cheese made from fortified milk, and with those of Yale and Marquardt (18) for *S. pyogenes.* The time of survival of *Brucella abortus* in Cheddar cheese is much longer than that obtained earlier by Wade and Shere (17) who manufactured Cheddar cheese from milk fortified with *E. typhosa* and found most of the bacteria dead in 36 days at 15.5° C.

The results obtained on commercial cheese milk and commercial cheese are especially interesting. In 1942, 15 vats of Cheddar-cheese milk were all negative. The 10 vats of Limburger-cheese milk contained two positives but the cheese curds from both positive milks were negative. In 1944, in the 34 vats of Cheddar cheese milk selected in areas in Wisconsin and New York where brucellosis had not been eradicated, there were nine positive for *Brucella abortus,* and the curd from one of the positive milks was negative. In the two surveys there were a total of 59 milk samples with 11 positives, but only eight positive cheese curds as taken from the vat. Of the cheeses made from positive milks nine were recovered for examination and all were negative on the first tests which were made after the cheeses had been stored for 41 to 84 days at 1.1° to 2.7° C. Fortunately, the two cheeses made from the most heavily infected raw milks were tested after 41 and 74 days aging. It is obvious, therefore, that *Brucella abortus* died much more rapidly in commercial cheese than in the fortified laboratory samples.

The question naturally arises as to the reason for the much more rapid death of *Brucella abortus* in commercial cheese as compared with inoculated laboratory samples. The obvious answer is that the commercial cheese had a very much lower initial contamination. This is indicated by the commercial manufacture of three lots of negative cheese curd from positive vats of milk. The evidence indicates that *Brucella abortus* did not multiply rapidly in the cheese milk and curd during the manufacturing process. If it had, the fresh curd in which the bacteria were held and concentrated should have contained more bacteria than the gravity cream from the milk used for guinea-pig injection. It should be pointed out that the size of the inoculum was less for commercial than for laboratory cheeses but in each case the dose was the maximum the animal could take subcutaneously.

The data indicate that aging for about 60 days is reasonable assurance of the absence of viable *Brucella abortus* in commercial Cheddar cheese. Cheddar cheese has not been proved to have produced undulant fever and most of this cheese under normal conditions has been consumed after two months of age. These results may not apply to the survival of *E. typhosa.* It may never be possible to definitely prove the time of survival of *E. typhosa* in commercial cheese except by the data on the limited numbers of cheese-borne typhoid epidemics. The evidence cannot be based upon cheese made from milk fortified with the pathogenic bacteria for such experiments would warrant the false conclusion that raw milk Cheddar cheese should be aged for one year to assure absence of *Brucella abortus* and *E. typhosa.* Except for the statement of Wade and Shere (17) that *E. typhosa* did survive in commercial Cheddar cheese for 63 days, the history of cheese-borne typhoid epidemics shows that no cheeses over 60 days of age were involved. The epidemiological evidence of Thomasson (13) is clear that *E. typhosa* survived in Colby type cheese for 45 days. Campbell and Gibbard (3) review the work of Bowman (1) and state that "evidence was presented indicating that the cheese remained dangerous for at least 80 days." Now Bowman (1) very carefully reported his findings on the typhoid fever epidemic due to cheese and he made no statement on the longevity of *E. typhosa* in Cheddar cheese for the data were not exact on this point. If the 80 days survival is to be accepted as proved, then one must know the identity of each cheese in the hands of a wholesaler who, Bowman stated, "kept no records," and the statement of Bowman that "we suspect that it was two cheeses from this vat No. 108" must be accepted as an established fact. The conclusions of Bowman are that cheese did cause the typhoid fever epidemic and that "either pasteurization of milk or adequate aging of cheese should be insisted upon.'' Bowman must have believed the evidence was not adequate to give data on the actual maximum survival of *E. typhosa* in Cheddar cheese for it was not mentioned by him.

The situation is well stated by Brooks (2) in his 1944 report of the committee on communicable diseases affecting man which was devoted to ''cheese-borne infection'' when he stated, ''If we assume that such terms as 'short-cured,' 'fresh,' 'green' and 'unripened' indicate storage for less than 60 days, a fairly safe assumption, then none of the outbreaks cited have been traced to cheese cured for this length of time.'' The experience of the epidemiologist with cheese-borne typhoid fever epidemics substantiates the data in this study on the survival of *Brucella abortus* in commercial Cheddar cheese that about 60 days aging is reasonable assurance of the absence of these viable pathogenic bacteria. An exception is *Brucella melitensis* in goat's milk cheese, as shown by Stiles (12), and goat's milk cheese presents a special problem.

CONCLUSIONS

Cheddar cheese was made from milk produced by a herd highly infected with brucellosis. The milk contained about 500 viable *Brucella abortus* bacteria per ml. and was then inoculated with a freshly isolated culture to contain about 1000 organisms per ml. *Brucella abortus* survived in the cheese for six months at 4.4° C. but the cheeses were all negative in one year as determined by guinea-pig inoculations.

When the milk was produced by reacting cows selected for eliminating large numbers of *Brucella abortus* the plate count was 700 to 800 *Brucella abortus* per ml. This pathogen remained viable in all the Cheddar cheeses made from such milk for three months, it could not be demonstrated in some cheeses in six months, and it was negative in all tests on guinea pigs in one year.

Tests were conducted on 10 vats of commercial cheese milk made into Limburger cheese and 15 vats made into Cheddar cheese produced in New York areas selected at random. There were two vats of Limburger-cheese milk positive for *Brucella abortus* with guinea pig inoculations and no Cheddar-cheese milk was positive. The Limburger curds made from the positive milks were negative and the cheese was negative on first test after 57 days storage.

Tests were also made on 34 vats of commercial Cheddar-cheese milk produced in areas in New York and Wisconsin where brucellosis had not been eradicated from the milking herds. There were nine positive milks and eight positive curds. Two cheeses were lost. The other seven cheeses made from positive milks were all free from viable *Brucella abortus* bacteria on the first examination as determined by guinea-pig inoculations. These tests were made after storage of the cheeses for 41 to 84 days at 1.1° to 2.7° C.

The data on all commercial cheeses gave a total of 59 vats of commercial cheese milk of which 11 were positive for *Brucella abortus*. There were three vats of positive milk that gave negative fresh curds. Two cheeses made from positive milks could not be located. The Cheddar cheeses from the nine positive milks were all negative on first examination after storage at 1.1° to 2.7° C. for periods varying from 41 to 84 days. No *Brucella abortus* organisms were recovered from any sample of commercial cheese.

It was pointed out that Cheddar cheese has not been proved to be a carrier for undulant fever and that reported typhoid fever epidemics have not been attributed to cheese cured for more than 63 days. These observations in the literature are of interest in considering the experimental data on commercial cheese in this study which show that an aging period of 60 days is reasonable assurance against the presence of viable *Brucella abortus* organisms in Cheddar cheese.

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THE CALCULATION OF TOTAL SOLIDS BY MEANS OF THE SHARP AND HART EQUATION

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In 1936, Sharp and Hart (4) published a careful study of the factors influencing the estimation of total milk solids in milk from measurements of specific gravity and fat content. As a result of their studies, they derived the equations:

$$\text{Total solids} = 1.2648\ \text{Fat} + 0.2586\ \text{Lactometer} \qquad \text{I}$$

and

$$\text{Total solids} = 1.2537\ \text{Fat} + 0.2680\,\frac{\text{Lactometer}}{\text{Sp. gr. milk}} \qquad \text{II}$$

The first equation is theoretically less accurate but more convenient to use. Since that time, several reports have reached the writer that the values calculated by Sharp and Hart's equation were too low. For example, a mimeographed "Report of Committee for Milk and Cream, American Dairy Science Association—June, 1943," contains the following statement:

> In the case of the Sharp procedure all laboratories found it to give results considerably lower than those obtained with the Mojonnier test. The average deviation ranging from -0.53 to -0.76%.

It is the purpose of this paper to point out one source of this error.

Sharp and Hart did not use a lactometer for their measurements. Their data were obtained by means of a modified Westphal balance and they recorded the specific gravity of milk as the ratio of the weights of equal volumes of milk and water when both were *at 30° C.*

They made an error when they attempted to convert these values to readings on a Quevenne lactometer. They overlooked the fact that their specific gravities were based upon water at 30° C. while the Quevenne lactometer gives specific gravities based upon water at 60° F. Values of specific gravity at 30° C./30° C. must be multiplied by 0.99663 to convert them to values at 30° C./60° F.

When a Quevenne lactometer is used at 30° C. it does not indicate exactly the specific gravity referred to water at 60° F., because there is an appreciable expansion of the lactometer when it is warmed from 60° F. to 30° C. If we assume that the value 0.00002648 given for the cubical coefficient of expansion of glass in the Handbook of Chemistry and Physics (1) is applicable to the ordinary lactometer, then we can convert true values of specific gravity at 30° C./60° F. to apparent specific gravities as measured with a lactometer, by multiplying by 1.00038. By multiplying these two correc-

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tion factors together, we can combine them into a single factor: 0.99701. Hence, the specific gravity data of Sharp and Hart can be converted to equivalent Quevenne readings at 30° C. by the formula

$$\text{Q.} = 1000(0.99701 \times \text{Sp. gr.} - 1.0000) \qquad \text{III}$$

from which

$$\text{Sp. gr. } 30°/30° = \frac{\text{Q.} + 1000}{997} \qquad \text{IV}$$

The Sharp and Hart equations may be rewritten in terms of the specific gravities at 30°/30° from which they were derived.

$$\text{Total solids} = 1.2648 \text{ Fat} + 258.6 \ (\text{Sp. gr.} - 1.0000) \qquad \text{V}$$

and

$$\text{Total solids} = 1.2537 \text{ Fat} + 268.0 \left(\frac{\text{Sp. gr.} - 1.0000}{\text{Sp. gr.}}\right) \qquad \text{VI}$$

Substituting the value of Sp. gr. (Equation 4) in the second term of equation 5 gives $\frac{258.6}{997}$ (Q. + 1000 − 997) which reduces to 0.2594 (Q. + 3). The complete corrected equation becomes

$$\text{Total solids} = 1.2648 \text{ Fat} + 0.2594 \ (\text{Q.} + 3) \qquad \text{VII}$$

In the case of equation VI two substitutions are necessary, because the Sp. gr. at 30°/30° appears twice in the equation. These substitutions yield the following corrected equation:

$$\text{Total solids} = 1.2537 \text{ Fat} + \frac{268.0 \ (\text{Q.} + 3)}{\text{Q.} + 1000}$$

If Quevenne lactometer readings are substituted directly into the original Sharp and Hart equation, the results for total solids will be about three-fourths of a per cent lower. This is approximately the error which has been observed by some users of the equation.

After the preceding paragraphs were written, two papers were found which deal with this same problem. Rueda (3) recognized that a correction must be applied to the ordinary lactometer readings before they could be used in the Sharp and Hart equation. He attempted to determine the magnitude of the correction by comparing the actual reading of a lactometer in a sucrose solution at 30° C. with the actual specific gravity at 30°/30° as determined by a pycnometer. In this way, he obtained a correction of 3.2° C. This differs by 0.2° Q. from the value calculated by the writer.

It is not clear from Rueda's paper whether he had checked the accuracy of his lactometer at 60° F. If not, then the small difference of 0.2° Q. might be due to a minor error in calibration of the lactometer. There are other possibilities. A hydrometer is affected by surface tension as well as by density. A liquid of higher surface tension will appear to have a lower density than another of identical density but lower surface tension. The difference of 0.2 might be due to differences in the surface tension of the sugar solution and of the solution used to check the accuracy of calibration of the lactometer. The reading of an ordinary Quevenne lactometer in pure water can be raised approximately four degrees by touching the surface of the water with a piece of soap.

Livak (2) calculated a correction based on the difference between the densities of water at 30° C. and at 60° F. Because he overlooked the effect of temperature upon the lactometer itself, he obtained a value of 3.5° Q. Livak went one step further and compared total solids calculated by a corrected Sharp and Hart equation with the results of Mojonnier analyses. These comparisons indicated that his correction of 3.5° and Rueda's of 3.2° were both too large. In order to make the calculated values agree with Mr. Livak's gravimetric values, it would be necessary to use a correction of approximately 2.3 in the Sharp and Hart equation.

It is not the purpose of the present writer to question or to defend the experimental work of either Livak or of Sharp and Hart which led to a difference of 0.7° Q. in the value of the correction. It is his intention only to point out the difference between actual lactometer readings and the calculated values used by Sharp and Hart, and to calculate the magnitude of this difference.

SUMMARY

There is an error in the derivation of the Sharp and Hart equations. By correcting this error, an equation is obtained which yields values for total solids which are approximately three-fourths per cent higher.

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THE VITAMIN A AND CAROTENE CONTENT OF MARKET BUTTER PRODUCED IN KANSAS[1]

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Great interest has arisen in the nutritional value of butter as a result of problems arising from the wartime food situation. In 1941 the Committee on Food and Nutrition of the National Research Council suggested to the Secretary of Agriculture that a study of the vitamin A potency of creamery butter should be undertaken. Through the cooperation of the Bureau of Dairy Industry and the Office of Experiment Stations a project was developed in which 20 experiment stations have participated in making a survey of the vitamin A potency of creamery butter produced in this country. This paper presents the results on butter produced in Kansas during the period July, 1943, through December, 1944. In addition to the determination of vitamin A and carotene, certain other data were obtained on butter composition and quality, pasture conditions and feeding practices, in an attempt to determine whether these factors are related to the vitamin A and carotene content of butter.

A review of the literature on this subject will be unnecessary since an excellent list of references relating to this problem has been published (7).

EXPERIMENTAL

Sampling. Butter was obtained from seven creameries located in different areas of the state (fig. 1). Six of these creameries, four of which are cooperatives, obtain cream from local sources. The seventh, Creamery F, is a large centralizer using cream from widely scattered sources. These seven creameries made about 20 per cent of the butter produced in Kansas during 1944. The samples were collected during the latter half of each month and represented small aliquots of each churning for the day samples were obtained. These samples, packed in small, tightly closed glass jars, were sent to the laboratory without refrigeration during transit. Except for a few samples sent during the hottest part of the summer, they arrived in a firm condition. Upon arrival the samples were placed in a refrigerator until analysis could be made. Probably no loss of vitamin occurred in handling butter in this manner since it has been shown that butter held for long periods under refrigeration or for even as much as three days at room temperature lost no vitamin A (2, 7). The samples were softened and mixed thoroughly before sampling for analysis.

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[1] Contribution No. 302, Department of Chemistry, and No. 161, Department of Dairy Husbandry.

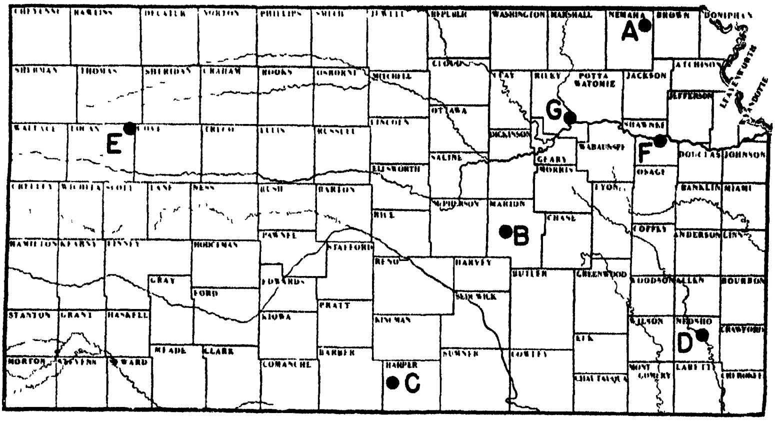

FIG. 1. Location of the seven cooperating creameries.

Methods of Analysis and Collection of Supplementary Data. Vitamin A and carotene were determined by methods developed under the direction of the Technical Committee on Vitamin A Researches (8, 9) with the following minor changes. A Coleman spectrophotometer, model 11, set at 620 mμ, was used for determining vitamin A by the Carr-Price reaction. During the course of this study it was discovered that the stability of the blue color was increased and the ease of reading the galvanometer was improved by decreasing the light intensity of the exciter lamp of the photometer through the use of a resistance unit (3). Carotene and total color (as carotene) were both read at 460 mμ. The standard calibration curves were essentially straight lines having the slopes shown by the K values in table 1.

The factor 0.005 was found to correct for the blue color produced by the reaction of antimony trichloride and carotenoids in concentrations of 2–10 micrograms per milliliter. This is similar to the concentrations of pigment encountered in the butter samples. Unlike the results reported by Jenness and Palmer (7), the slope of the calibration curve of the β-carotene and

TABLE 1

Calibration of the Coleman spectrophotometer for vitamin A analysis

Substance	Solvent	Range of concentration	Wave length	*K**
		μg./ml.	*mμ*	
Vitamin A alcohol (Dist. Prod. Co.)	Chloroform	1.0–6.0	620	14.6
β-Carotene (S.M.A.)	"	0.2–2.5	460	3.35
β-Carotene (S.M.A.)	Skellysolve B	0.2–2.5	460	3.0

* $K = \frac{\mu g./ml.}{2 - \log G}$ (for vitamin A); $K = \frac{\mu g./ml.}{\text{optical density}}$ (for β-carotene). G is the galvanometer reading with the galvanometer previously set at 100 on a mixture of 9 ml. of antimony trichloride reagent and 1 ml. of chloroform.

antimony trichloride reaction-mixture was found to increase rapidly at higher concentrations; however, fairly satisfactory results were obtained for the concentrations used in this study. Methods for making calibration curves and determining the correction factor have been described elsewhere (4, 9, 10).

The color of the butter was compared to the Nafis standard, and it was scored for quality in the usual manner.

Data on butter production were obtained from the Dairy Commissioner of Kansas and the reports of the Bureau of Agricultural Economics of the United States Department of Agriculture. Pasture conditions, feeding practices, etc., were supplied by county agents of the areas in which the creameries were located.

RESULTS

Content and Variation in Vitamin A Potency of Kansas Butter. The values for the vitamin A and carotene content of butter included in this study are shown in table 2. The values are weighted for the monthly production of each of the cooperating creameries. The factors used to convert the values to International Units will be discussed in another section of this report. The correction made for vitamin A lost in analysis is based on a recovery of 93 per cent when vitamin A was added to butter before analysis.[2]

The mean vitamin A potency for the 1944 "winter" butter was 11,050 I.U. per pound, compared to 17,700 I.U. per pound for the "summer" months, and 15,100 I.U. per pound for the mean annual value. The values for corresponding months of 1943 and 1944 (table 2) show the vitamin A potency was appreciably higher in 1944 for the months of September, October, and November. In the fall of 1943 the grass was not as good as in the fall of 1944 when plentiful rainfall and mild weather produced particularly good pasture. In some parts of the state abundant cereal grass pasture was available until late in the year.

The mean annual vitamin A potency is slightly higher than the value reported by Jenness and Palmer from Minnesota (7), but the difference is unimportant since they apparently made no correction for vitamin A lost in analysis.

The vitamin A potency of Kansas butter was lowest in January and February (values of 9,350 and 8,950 I.U. per pound respectively), but increased rapidly from April to June, reaching a high of 20,000 I.U. per pound. Through July and August the values dropped off somewhat but again increased in the fall. The values for both vitamin A and carotene follow the same general trend, although some variation in the relative positions occur from month to month, and the change for vitamin A was less than the change for carotene. The mean carotene content increased from 1.63 micrograms per gram in February to 5.25 micrograms per gram in June, the latter value being 3.2 times higher. Even slightly greater values were obtained in November (table 2). In the same months the vitamin A values were 3.95 and 8.2 micrograms per gram, or 2.1 times higher in June. In Minnesota during 1943, almost a fourfold change was found in the carotene content of the butter from the fourth to the sixth 4-week sampling periods.

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[2] C. A. Cary. Unpublished communication.

TABLE 2

The vitamin A potency of Kansas market butter[a]

Year and month of production	Distribution of annual butter production	Carotene per gram	Vitamin A per gram	Potency per pound[b]		Vitamin A potency due to carotene
				Not corrected for loss in analysis	Corrected for loss[c] of vitamin A in analysis	
	% per mo.	*µg.*	*µg.*	*I.U.*	*I.U.*	*% of total*
1943:						
July	10.13	5.61	5.92	15,000	15,800	26.8
August	8.55	5.32	4.88	12,900	13,550	29.6
September	6.82	3.62	6.33	14,200	15,100	18.2
October	5.94	3.53	5.44	12,550	13,300	20.0
November	6.15	3.28	4.94	11,500	12,100	20.4
December	6.04	2.42	4.69	10,350	11,000	16.6
1944:						
January	7.25	1.86	4.07	8,800	9,350	15.0
February	8.21	1.63	3.95	8,400	8,950	13.8
March	9.11	1.74	5.15	10,700	11,400	11.5
April	9.16	2.65	6.07	13,050	13,850	14.5
May	11.63	5.02	8.15	18,600	19,750	19.3
June	11.16	5.25	8.2	18,850	20,000	19.9
July	10.18	5.03	6.19	15,050	15,950	23.9
August	8.82	4.71	6.25	14,900	15,800	22.6
September	7.13	4.70	6.67	15,700	16,600	21.5
October	5.83	4.88	6.89	16,200	17,150	21.6
November	6.02	5.51	6.69	16,300	17,250	24.2
December	5.73	3.32	4.44	10,600	11,300	22.4
Mean, July, 1943, to June, 1944[d]				13,370	14,170	19.8
Mean, December to April, 1944[d, e]				10,400	11,050	14.9
Mean, May to November, 1944[d, f]				16,800	17,700	21.4
Mean. annual. 1944[d]				14.250	15.100	19.6

[a] All values represent the mean, weighted according to production for the seven creameries sampled.

[b] 0.6 microgram carotene = 1 I.U.; 1 microgram of vitamin A = 4 I.U.

[c] Corrected for loss in analysis, based on recovery of 93 per cent of vitamin A.

[d] Weighted for monthly production.

[e] Production for December to April, inclusive, was 39.2 per cent of the annual output.

[f] Production for May to November, inclusive, was 60.8 per cent of the annual output.

The low and high vitamin A values were observed in the third and ninth periods, the value being about 1.9 times higher during the latter period (7).

The trends in the vitamin A potency can be seen to follow the seasonal and pasture conditions. In January, February, and often into March little pasture is available over most of the state. But with the approach of spring both cereal and native grass pastures develop rapidly. The carotene and vitamin A values of the butter clearly reflect this change in feeding conditions. The drop in the values during July and August is probably due to the effect of hot weather. In areas of low rainfall the pastures rapidly deteriorate during hot weather. However, the possibility that a part of the decrease in the vitamin content may be due to the effects of weather on the milk, cream, or butter cannot be overlooked.

The values shown for 1944 follow a trend similar to those found by Jenness and Palmer for corresponding months of 1943 (7), although Kansas values show appreciable increases in March and April which are not shown in Minnesota. While the Minnesota values in the fall were fully as high as in the spring, there were considerable decreases during the last two 4-week sampling periods of the year. The values obtained in this study were high for September, October, and November but dropped off sharply in December. The geographical locations of the two states suggest an explanation for these results. No doubt milder winters, lasting for a shorter length of time,

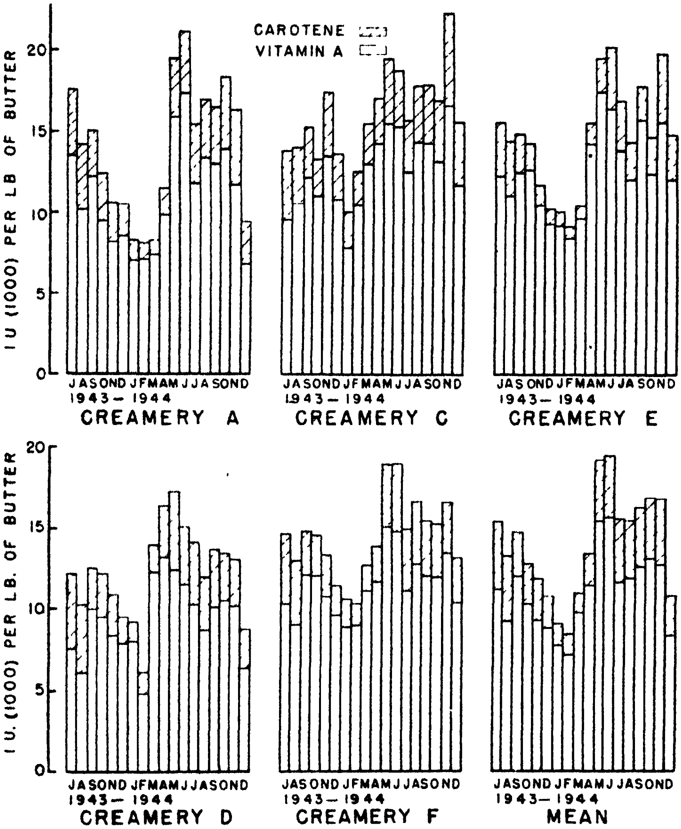

FIG. 2. Monthly variations in vitamin A potency due to carotene and vitamin A. Letters beneath the bars indicate the months. The mean represents values which have been weighted for the production of each of the seven creameries included in this study.

makes pasture available in Kansas for a greater part of the year. The cereal grasses are generally available for pasturage early in the spring and late in the fall, providing an excellent source of carotene in addition to the native grasses and dry roughage. In fact, in many parts of the state when the weather is mild, occasional use may be made of wheat pasture during the winter months.

TABLE 3

Mean vitamin A potency of butter produced by seven Kansas creameries in 1944

	Carotene	Vitamin A*	Potency due to carotene	Total vitamin A potency*
	I.U./lb.	*I.U./lb.*	*% of total*	*I.U./lb.*
Creamery A				
Mean, Dec. to Apr.	1,500	8,050	15.7	9,560
Mean, May to Nov.	3,900	14,400	21.2	18,400
Mean, annual	2,900	11,800	19.7	14,700
Creamery B				
Mean, Dec. to Apr.	1,200	8,600	12.3	9,800
Mean, May to Nov.	3,700	14,400	20.2	18,200
Mean, annual	2,670	12,000	18.2	14,700
Creamery C				
Mean, Dec. to Apr.	2,730	12,050	18.5	14,800
Mean, May to Nov.	3,940	15,000	20.8	18,950
Mean, annual	3,460	13,850	20.0	17,300
Creamery D				
Mean, Dec. to Apr.	2,020	9,750	17.2	11,800
Mean, May to Nov.	3,750	11,000	25.4	14,700
Mean, annual	3,140	10,550	23.0	13,700
Creamery E				
Mean, Dec. to Apr.	1,140	11,000	9.4	12,200
Mean, May to Nov.	2,970	15,600	16.0	18,550
Mean, annual	2,170	13,550	13.8	15,700
Creamery F				
Mean, Dec. to Apr.	1,910	10,650	15.2	12,600
Mean, May to Nov.	3,740	13,600	21.6	17,350
Mean, annual	3,020	12,450	19.5	15,500
Creamery G				
Mean, Dec. to Apr.	1,450	9,250	13.6	10,700
Mean, May to Nov.	4,020	14,700	21.4	18,700
Mean, annual	2,790	11,900	18.5	14,600
Mean of seven creameries†				
Mean, Dec. to Apr.	1,640	9,400	14.9	11,050
Mean, May to Nov.	3,800	13,900	21.4	17,700
Mean, annual	2,950	12,100	19.6	15,100

* Corrected for loss in analysis, based on recovery of 93 per cent vitamin A.
† Weighted for production.

The variations in the vitamin A, carotene, and total vitamin A potency of butter produced in the different areas is shown in table 3. Butter from creamery C, located in the southern part of the state where considerable use is made of cereal grass pasture, had the highest "winter" as well as the highest annual vitamin A potency. The "summer" mean is not appreciably higher than that of the butter from several other creameries. This creamery also makes a butter of considerably higher carotene content in the winter months than was found for creamery A in the northeastern or creamery E in the northwestern parts of the state (fig. 2). But the differences in the carotene content of the butters from these three creameries tend to vary much less in the summer and fall months. The availability of pasture in the different areas is probably the explanation for these results.

Creamery D, in the southeastern area, produced butter of the lowest annual mean vitamin A potency. It is interesting to note that butter from this source contains a higher per cent of the total vitamin A potency as carotene than does butter from any other creamery except creamery C during the "winter" period (table 3). A greater percentage of cows of the Jersey breed are found in this area than in any of the others included in this study.

During the winter the percentage of the total vitamin A potency due to carotene is less than during the summer months. The mean value is 14.9 per cent in the "winter" and 21.4 per cent in the "summer" months. Carotene accounted for 6.5 per cent of the vitamin A potency of the March butter from creamery E and 27.8 per cent of the vitamin A potency of the November butter made by creamery A. These values represent the extreme variations of potency due to carotene as found in this study.

An attempt was made to correlate the Nafis color grade with the vitamin content of the butter. While there was a tendency for the colors to be a little lighter in the winter months, the use of artificial colors made impossible a meaningful correlation study. All except two of the samples of butter graded either A or AA by the Nafis color standards.

No correlation was found between butter score and vitamin A potency. These findings agree with those previously reported (2).

DISCUSSION

Notes on the Factors for Converting the Vitamin A and Carotene to Units of Potency and on Nutritional Significance. The potency of 0.6 micrograms of β-carotene has been adopted as 1 I.U. (6). However, there is still considerable question about the conversion factor for vitamin A. Baxter and Robeson found that 1 microgram of vitamin A averages 4.3 U.S.P. Units (1). Although U.S.P. Units and International Units were originally set as equivalent to each other, there is evidence of variability and loss of potency in the U.S.P. Reference Oil[3] (11). Gridgeman recently has summarized the work of a number of investigators which deals with these conversion factors (5). The use of 1 microgram of vitamin A as equivalent to 4 I.U. has been adopted for the results reported in this paper. This is probably the most satisfactory factor available at the present time. Since this factor already has been adopted by other laboratories participating in this study of butter (2, 7), the results of the different investigations may be compared.

The rat has been used as the test animal for establishing the relationship

[3] H. G. Wiseman. Personal communication.

of β-carotene and vitamin A to units of potency. The factor which will represent the conversion of these substances to units of vitamin A potency by humans has not been investigated sufficiently to warrant the use of a value more refined than that found in the rat-feeding investigations.

The material determined as β-carotene by the methods employed in this investigation may include other carotenoids as well as optical isomers of β-carotene. Variations in these constituents may cause the true vitamin A potency of butter to differ by a few per cent from the values found by analysis.

The limitations of the factors for converting vitamin A and carotene to units of vitamin A potency should be realized, especially when interpreting these results in terms of the requirements for human nutrition.

The results of the study of the vitamin A potency of butter reported to date indicate butter is probably a richer source of vitamin A than has been shown by many of the older nutrition tables which frequently give average values of 9,000–10,000 I.U. per pound. This study indicates Kansas butter has a mean potency possibly 50 per cent higher than this value.

It should be noted that in the late spring and early summer, a period of high vitamin A potency, more butter is produced than in the winter months when the vitamin A potency is lowest (table 2). Normally considerable quantities of butter go into storage during months of high production and will be placed on the market when the demand is greater. No loss of vitamin A potency has been found to occur when butter is stored under refrigeration (7), and the consumer may obtain butter of high vitamin A potency during periods when the butter being produced is of lower vitamin content. Since this condition exists, the mean annual vitamin A potency of butter probably represents the best indication of what the consumer obtains when buying butter for dietary requirements.

SUMMARY

1. The 1944 mean annual vitamin A potency of Kansas butter was 15,100 I.U. per pound. The mean for the period December to April was 11,050 I.U. per pound, and from May to November, 17,700 I.U. per pound.

2. In the above periods carotene accounted for 19.6 per cent, 14.9 per cent, and 21.4 per cent of the total vitamin A potency of Kansas butter.

3. The vitamin A and carotene content of butter produced in different areas of the state and during different months of the year varied with the pasture available. When little or no pasture existed the values for vitamin A and carotene dropped. Good pasture conditions increased the vitamin A and carotene values to maximum in May and June. During the hot summer weather the values dropped off somewhat, returning to higher levels when pastures improved in the fall of the year.

4. No correlation was found between the Nafis color grade or butter score and the vitamin A potency of butter.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the creameries supplying the butter samples for this study and to Professor W. J. Caulfield who assisted with the collection and scoring of part of the samples.

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SOME OBSERVATIONS ON THE ACID INVERSION OF SUCROSE

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When sugar rationing went into effect early in 1942, the dairy manufacturing industry, particularly ice cream manufacturers, became concerned about supplies of sweetening materials. Among other ingredients which evoked the interest of ice cream manufacturers, invert sugar sirup received considerable attention. During the ensuing two-year period there appeared in technical and semi-technical literature a great deal of controversial discussion concerning the relative sweetness of invert sugar sirup. Very few of these discussions were based upon experimental data.

Of all the probable factors related to sweetness of invert sirup, apparently no consideration has been given to variations in the composition of the sirup resulting when the inversion process is not carefully controlled. It has been tacitly assumed that the sucrose is always completely hydrolyzed during inversion, and that the resulting "invert" sugar is always composed of exactly equal quantities of dextrose and levulose (as theoretically it should be).

It was thought that a systematic study of some of the factors affecting the inversion of sucrose might aid in resolving some of the confusion concerning the sweetness of invert sugar. No organoleptic sweetness comparisons were made in this investigation, the primary object being to obtain some basic information on the applied chemistry of sucrose inversion. Furthermore, it was hoped that these experiments might reveal procedures for making invert sirup that would be more widely applicable than methods which have been recommended heretofore.

REVIEW OF LITERATURE

Very little detailed information is available relative to the effect on the inversion of sucrose of such factors as kind of acid used, pH, temperature employed, and time of holding at the hydrolyzing temperature. These are all conditions which may, either intentionally or inadvertently, be varied from one batch to another.

Taussig (6, 7) recommended boiling a solution of sugar and citric acid to a temperature of 232.5° F., followed by sudden cooling. He stated also that satisfactory results could be obtained with a temperature of 210°–218° F. maintained for an hour, followed by raising the temperature to the point "just below caramelization" then sudden cooling.

Baudrexel (2) reported boiling for 15 to 30 minutes a solution consisting of 100 kg. sucrose, 27–30 kg. water, 0.5 kg. $CaCl_2$, 1 kg. NaCl, and 300 g. citric acid (or 150–250 g. tartaric acid).

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In 1929 Schaal and Johnson (5) reported that beet sugar required more acid than cane sugar for satisfactory inversion because of the greater buffering capacity of the beet sugar. Their recommended procedure was to boil the sugar-water solution for 30 minutes then cool immediately. They found that for the above procedure the optimum pH was about 2.5. These workers apparently considered that approximately 90 per cent inversion was satisfactory in the production of commercial invert sirup.

EXPERIMENTAL PROCEDURE

The investigation reported in this paper was divided into four phases, as follows: *A*—Effect of kind of acid and pH on sucrose inversion. *B*—Effect of concentration of solution on sucrose inversion. *C*—Effect of boiling time on sucrose inversion. *D*—Time, temperature, and pH as factors affecting sucrose inversion.

Cane sugar was employed as the source of sucrose in the experiments reported herein.

A. Effect of kind of acid and pH on sucrose inversion. The acids used in this portion of the study were tartaric, citric, lactic, acetic, hydrochloric, sulfuric, and phosphoric. From each acid was prepared a solution of suitable concentration for obtaining the desired pH range when the acid solution was added to the sirup in varying quantities. The following amounts of acid in 100 ml. of solution were found to be satisfactory: tartaric, 30 grams; citric, 20 grams; lactic, undiluted; acetic (glacial), undiluted; hydrochloric, 3 ml. concentrated; sulfuric, 2 ml. concentrated; phosphoric, 3 ml. of 85%. The amounts of these solutions used are indicated in the data tables given under "Results."

All sirups were prepared by mixing together 1 lb. sucrose, 0.44 lb. water, and the required amount of acid solution. These solutions were heated quickly to boiling in a 1-liter beaker by placing on a wire gauze over a Bunsen burner. Gentle boiling was maintained for 30 minutes, the beaker being covered with a watch glass to reduce evaporation. The beaker of sirup was then removed from the burner and placed on the table top to allow it to cool to room temperature. Any measurable loss of water by evaporation was then restored. The water thus restored was mixed thoroughly into the sirup, which was then sampled for analysis.

Analyses conducted on each sample of sirup consisted of a pH determination and an assay of the dextrose and levulose content. The pH values were obtained with a glass electrode, employing a Leeds and Northrup Potentiometer-Electrometer for making the measurements. For ascertaining the amount of dextrose and levulose present in the sirup, the iodine oxidation method described by Lothrop and Holmes (4) was employed. In this method it is suggested that any satisfactory procedure for determining total reducing sugar may be used. The writer chose to use the Lane-Eynon volumetric procedure (1).

B. Effect of concentration of solution on sucrose inversion. Five batches of invert sirup were prepared by using 1 lb. sucrose and varying amounts of water. The amounts of water used were 175 ml., 200 ml., 225 ml., 250 ml., and 300 ml. Tartaric acid was used as the catalyst. The sirups were boiled for 30 minutes in 1-liter Erlenmeyer flasks. Each flask was provided with a thermometer and a glass tube 7 mm. x 30 inches, which served as an air-cooled reflux condenser to minimize loss of moisture. Analyses for pH, dextrose content, and levulose content were made according to methods described under A.

C. Effect of boiling time on suucrose inversion. Three series of sirup were prepared in this phase of the study. In each series the sirups were prepared by boiling together 1 lb. sucrose and 0.44 lb. of water, using tartaric acid as the catalyst.

In series I, one batch of sirup was boiled under a reflux condenser. Samples for analysis were withdrawn at the end of 20 minutes, 30 minutes, and 40 minutes.

In series II, three separate batches of sirup were prepared, using the same proportions of ingredients as in series I. The three batches were boiled under reflux condensers for 15 minutes, 30 minutes, and 45 minutes, respectively.

Series III was a repetition and extension of Series II. Four batches of sirup were prepared by boiling for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, respectively.

Analyses of all sirups for pH, dextrose content, and levulose content were made according to methods described under A.

D. Time, temperature, and pH as factors affecting sucrose inversion. Temperatures used in this phase of the study were 190° F. and 175° F. At 190° F. the sirups were held for 30 minutes, 45 minutes, 60 minutes, and 90 minutes. At 175° F., heating periods of 45 minutes, 60 minutes, 90 minutes, and 120 minutes were employed. Five or more batches of sirup were prepared at each temperature and holding time, the variable factor being pH. Two parallel series of sirups representing the above combinations of conditions were made (82 batches of sirup), using tartaric acid in one series and phosphoric acid in the other.

Analyses of all sirups for pH, dextrose content, and levulose content were made according to methods described under A.

RESULTS

The relationship between the kind and amount of acid used and the results of the inversion process is indicated by the data shown in table 1. In connection with the values for the "total reducing sugar" it might be pointed out that, by way of comparison, the sirup should theoretically contain 73.07 per cent total reducing sugar if the sucrose were completely inverted, and the resulting invert sugar completely recovered.

TABLE 1

Effect of kind of acid and pH on sucrose inversion

Acid solution used	pH of sirup	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical sweetness index
		Tartaric acid			
ml.		*per cent*	*per cent*	*per cent*	
0.25	3.20	22.86	21.51	44.37	65.88
0.50	2.83	34.22	29.50	63.72	93.22
1.50	2.54	36.00	29.41	65.41	94.82
5.0	2.09	36.05	33.09	70.14	103.23
20.0	1.73	35.69	30.42	66.11	96.53
		Citric acid			
0.50	3.41	19.38	19.31	38.69	58.00
1.0	3.00	31.24	26.26	57.50	83.76
2.0	2.77	35.06	30.03	65.09	95.12
12.0	2.15	36.03	34.45	69.48	103.93
6 grams*	1.91	34.53	32.31	66.84	99.15
		Lactic acid			
0.20	3.16	23.86	22.04	45.90	67.94
0.75	2.68	34.47	32.24	66.71	98.95
1.50	2.45	35.85	30.64	66.49	97.13
3.0	2.23	36.61	32.83	69.44	102.27
6.0	2.00	35.86	33.41	69.27	102.68
14.0	1.79	35.41	31.51	66.92	98.43
		Acetic acid			
0.50	3.40	16.37	15.11	31.48	46.59
3.0	2.80	33.96	32.81	66.77	99.58
15.0	2.25	35.63	35.16	70.79	105.95
25.0	2.20	35.41	36.50	71.91	108.41
40.0	2.03	35.64	34.09	69.73	103.82
55.0	1.91	34.82	32.19	67.01	99.20
		Hydrochloric acid			
1.0	3.75	7.59	5.29	12.88	18.17
2.0	2.63	35.96	33.71	69.67	103.38
3.0	2.17	35.68	32.25	67.93	100.18
5.0	1.85	34.98	30.98	65.96	96.94
10.0	1.52	32.38	25.57	57.95	83.52
		Sulfuric acid			
0.75	3.20	16.84	17.57	34.41	51.98
1.00	2.71	32.12	32.91	65.03	97.94
1.50	2.43	35.66	33.97	69.63	103.60
2.50	2.03	35.35	33.36	68.71	102.07
5.0	1.65	34.34	30.54	64.88	95.42
		Phosphoric acid			
1.00	3.24	17.75	16.78	34.53	51.31
2.00	2.55	35.33	31.17	66.50	97.67
4.0	2.18	35.27	31.70	66.97	98.67
7.0	1.85	35.18	33.05	68.23	101.28
15.0	1.61	35.36	28.63	63.99	92.62

* Pure crystals.

TABLE 2

Effect of concentration of solution on sucrose inversion

Batch No.	Amount water per pound sucrose	pH		Dextrose in sirup			Levulose in sirup			Total reducing sugar in sirup			Theoretical Sweetness Index		
	ml.			*per cent*			*per cent*			*per cent*					
		A	B	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
1	175	2.10	2.05	35.27	35.66	35.47	28.91	30.87	29.89	64.18	66.53	65.36	93.09	97.40	95.25
2	200	2.11	2.07	35.23	35.96	35.60	32.59	30.20	31.40	67.82	66.16	66.99	100.41	96.36	98.39
3	225	2.13	2.09	35.28	35.96	35.62	32.21	32.20	32.21	67.49	68.16	67.83	99.70	100.36	100.04
4	250	2.18	2.13	35.60	36.09	35.85	33.21	33.96	33.59	68.81	70.05	69.43	102.02	104.01	103.02
5	300	2.22	2.18	35.32	36.22	35.77	34.55	31.84	33.20	69.87	68.06	68.97	104.42	99.90	102.17

A word of explanation is also necessary regarding the "Theoretical Sweetness Index" values tabulated in the last column of table 1. This is a purely arbitrary value which is the sum of the per cent total reducing sugar and per cent levulose in the invert sirup. It was believed that the total reducing sugar content alone, notwithstanding its importance, would not give a satisfactory sweetness value for comparing different methods of inverting sucrose. Theoretically at least, a high proportion of levulose in the invert sirup would be necessary to obtain maximum sweetness. For want of a better means, then, the "Theoretical Sweetness Index" was adopted for the purpose of permitting a quantitative comparison to be made of the theoretical sweetness of sirups produced by the various procedures considered in this paper.

TABLE 3

Effect of boiling time on sucrose inversion

Boiling time	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		(a)		
min.	%	%	%	
20	35.72	31.19	66.91	98.10
30	36.14	34.58	70.72	105.30
40	35.95	33.18	69.13	102.31
		(b)		
15	34.92	29.36	64.28	93.64
30	34.60	33.09	67.69	100.78
45	34.27	33.66	67.93	101.35
		(c)		
15	34.13	35.20	69.33	104.53
30	35.84	32.72	68.56	101.28
45	35.69	31.11	66.80	97.91
60	35.45	30.87	66.32	97.19

Note: The pH of the above sirups was approximately 2.1 to 2.2.

In the commercial preparation of invert sirup 95 per cent inversion is considered satisfactory. If, then, 95 per cent of the sucrose is inverted and the resulting invert sugar consists of equal parts of dextrose and levulose, the Theoretical Sweetness Index (TSI) would be $(0.95 \times 73.07) + \frac{0.95 \times 73.07}{2}$, or 104.13. For purposes of comparison a TSI of 104 appears to be a reasonable standard value.

The effect of sugar concentration on the inversion process is indicated by the data given in table 2. Two series of sirups, A and B, were prepared separately in the endeavor to obtain more representative results. In order to simplify a comparison of the five batches of sirup, all analytical values for dextrose, levulose, and total reducing sugar were adjusted to an equivalent water content. All data in table 2 were recalculated on the basis of 200 ml. water (Batch No. 2), so that the figures would be comparable to those given in table 1.

In table 3 are given the data showing the effect of the length of the boiling period on the inversion of sucrose. In this table parts (a), (b), and (c) correspond to Series I, II, and III, respectively, as outlined in *Experimental Procedure, C.*

Because of the large quantity of data secured in the investigation of the effect of temperature and time on sucrose inversion, these results are presented in four parts, namely, tables 4, 5, 6, and 7.

TABLE 4

Effect of pH and heating time on inversion of sucrose at 190° F. using tartaric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
30-minute heating				
	%	%	%	
3.37	3.16	2.94	6.10	9.04
3.03	5.58	3.86	9.44	13.30
2.58	18.15	14.07	32.22	46.29
2.11	30.35	28.53	58.88	87.41
1.67	32.88	37.12	70.00	107.12
45-minute heating				
3.18	6.69	3.52	10.21	13.73
2.97	11.90	7.08	18.98	26.06
2.48	25.11	16.04	41.15	57.19
2.06	34.10	32.05	66.15	98.20
1.67	35.45	34.57	70.02	104.59
60-minute heating				
3.21	9.44	3.88	13.32	17.20
2.92	15.62	7.92	23.54	31.46
2.46	28.60	20.98	49.58	70.56
2.07	34.65	29.29	63.94	93.23
1.67	35.55	33.71	69.26	102.97
90-minute heating				
3.31	9.69	6.44	16.13	22.57
2.92	18.90	14.78	33.68	48.46
2.49	31.20	28.32	59.52	87.84
2.08	35.54	34.74	70.28	105.02
1.70	35.69	30.19	65.88	96.07

DISCUSSION

A. Effect of kind of acid and pH on sucrose inversion. It will be helpful, in studying the data presented in the foregoing tables, to keep in mind the results which one is supposed to obtain by inverting sucrose. By a perfect inversion of sucrose solutions of the concentration used in this study, theoretically one should obtain a sirup containing 73.07 per cent total reducing sugar, half of which (36.54 per cent of the sirup) would be levulose and the other half dextrose. The Theoretical Sweetness Index would be 109.61.

Examination of table 1 reveals at once that this accomplishment was not realized in one of the 37 batches of sirup. The nearest approach (98.4 per cent) to "complete" inversion was in the batch of sirup prepared with acetic acid at a pH of 2.20. Likewise the highest TSI was obtained in this batch. Ironically enough, the acetic acid sirups were the only ones which were not usable in ice cream because of the undesirable penetrating aroma of the acid.

TABLE 5

Effect of pH and heating time on inversion of sucrose at 190° F. using phosphoric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		30-minute heating		
	%	%	%	
3.43	4.30	1.19	5.49	6.68
2.68	15.49	8.96	24.45	33.41
2.13	30.42	23.21	53.63	76.84
1.87	34.39	27.76	62.15	89.91
1.63	35.36	34.71	70.07	104.78
		45-minute heating		
3.53	4.06	2.39	6.45	8.84
2.72	18.17	12.93	31.10	44.03
2.13	32.37	24.94	57.31	82.25
1.88	34.88	34.03	68.91	102.94
1.63	36.43	33.67	70.10	103.77
		60-minute heating		
3.52	4.82	2.85	7.67	10.52
2.75	16.61	17.04	33.65	50.69
2.13	33.45	26.53	59.98	86.51
1.89	35.35	30.18	65.53	95.71
1.67	35.43	35.37	70.80	106.17
		90-minute heating		
3.53	6.26	7.43	13.69	21.12
2.72	22.59	23.60	46.19	69.79
2.17	34.13	35.47	69.70	105.17
1.92	35.56	35.34	70.90	106.24
1.67	35.46	35.73	71.19	106.92

It will be noted that in 34 of the 37 batches of sirup the quantity of dextrose found exceeded that of levulose—in many cases by an appreciable margin. The levulose content also appears to be somewhat more variable than the dextrose content. This relationship accounts for the fact that, in each series of sirups, the highest total reducing sugar content and the highest

TSI are usually found in that batch which contains the largest quantity of levulose.

Judging from the data presented in table 1 it appears that the optimum pH for inverting sucrose at the boiling temperature is within the range 2.0 to 2.6, and that the stronger the acid being used, the higher the pH should be. Careful study of the data in tables 1, 4, 5, 6, and 7 suggests that the pH of the sirup is a very important factor in determining the consequences of the inversion process.

TABLE 6

Effect of pH and heating time on inversion of sucrose at 175° F. using tartaric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		45-minute heating		
	%	%	%	
3.16	4.85	8.00	12.85	20.85
2.86	8.52	15.36	23.88	39.24
2.43	18.03	30.35	48.38	78.73
2.13	25.87	34.12	59.99	94.11
1.68	35.20	36.10	71.30	107.40
		60-minute heating		
3.20	5.94	7.43	13.37	20.80
2.89	10.56	12.85	23.41	36.26
2.46	20.89	21.90	42.79	64.69
2.08	31.63	32.76	64.39	97.15
1.74	35.26	35.75	71.01	106.76
		90-minute heating		
3.53	3.01	4.17	7.18	11.35
3.16	6.72	10.42	17.14	27.56
2.60	17.06	26.78	43.84	70.62
2.16	30.46	35.46	65.92	101.38
1.73	35.43	36.03	71.46	107.49
		120-minute heating		
3.47	4.25	5.63	9.88	15.51
3.13	8.03	11.46	19.49	30.95
2.55	22.31	27.23	49.54	76.77
2.10	32.77	36.58	69.35	105.93
1.71	35.47	36.72	72.19	108.91

At this point it should be mentioned that in each acid series reported in table 1, what appears to be the optimum pH is merely the optimum of the pH values studied. Taking the citric acid series as an example, a pH of approximately 2.15 may or may not be optimum. All that is known from the data available is that somewhere within the pH range 2.77 to 1.91 one should obtain best results. Further investigation along this line would undoubtedly yield interesting information.

The practical results of this phase of the study would seem to be that a reasonably good invert sirup can be made by the boiling procedure, using any of the acids employed in these experiments (except acetic acid), assuming that proper control of pH in the sirup is exercised.

B. Effect of concentration of solution on sucrose inversion. It has been recommended in the past that invert sirup for ice cream manufacture be prepared from a sucrose solution consisting of 100 pounds of sucrose and 44 pounds of water. This ratio of sugar to water was no doubt selected so that one pound of invert could be used to replace one pound of sucrose in the ice cream mix, thus simplifying mix calculations.

TABLE 7

Effect of pH and heating time on inversion of sucrose at 175° F. using phosphoric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
45-minute heating				
	%	%	%	
3.46	2.02	3.51	5.53	9.04
2.75	6.49	15.39	21.88	37.27
2.17	20.35	36.02	56.37	92.39
1.91	28.67	30.30	58.97	89.27
1.82	29.28	32.06	61.34	93.40
1.68	33.24	33.43	66.67	100.10
60-minute heating				
3.57	2.32	3.79	6.11	9.90
2.77	9.69	18.89	28.58	47.47
2.18	21.21	22.69	43.90	66.59
1.89	30.36	30.21	60.57	90.78
1.65	34.26	34.05	68.31	102.36
90-minute heating				
3.48	3.05	4.77	7.82	12.59
2.69	12.68	20.44	33.12	53.56
2.13	26.52	35.89	62.41	98.30
1.85	32.89	36.28	69.17	105.45
1.68	35.18	36.70	71.88	108.58
120-minute heating				
3.74	2.51	3.26	5.77	9.03
2.85	12.18	19.04	31.22	50.26
2.17	29.00	35.63	64.63	100.26
1.90	33.97	33.21	67.18	100.39
1.69	35.15	34.94	70.09	105.03

The TSI values in table 2 show that as the proportion of water was increased from 27.5 per cent (Batch 1) to 35.1 per cent (Batch 4) there was a gradual improvement in the inversion process. This was due primarily to an increase in the levulose content, for there was no significant difference among the several batches of sirup with respect to dextrose content. It is questionable whether the better inversion in the less concentrated solution is of much practical importance, inasmuch as the disadvantage of having to use a more dilute sirup would at least partly offset the advantage of slightly better inversion.

C. Effect of boiling time on sucrose inversion. It is to be noted that there is some difference in the results obtained with the three series of samples reported in this phase of the study. The data in table 3a (Series I) indicate that 30 minutes boiling time is definitely superior to either 20 minutes or 40 minutes. Either 30 minutes or 45 minutes boiling was much superior to 15 minutes in the Series II sirups. The advantage of boiling 45 minutes as compared with 30 minutes is probably too small in this instance to be considered significant. In the Series III trials the 15-minute period seemed to give somewhat better results than the three longer boiling periods. It is difficult to account for what appears to be an anomalous behavior of the inversion process in this series.

The data of table 3 substantiate previous observations that the variations in the TSI are principally the result of variations in the levulose content of the sirups. The dextrose content of the sirups prepared by the different boiling periods exhibit a remarkable degree of uniformity.

D. Time, temperature, and pH as factors affecting sucrose inversion. A number of interesting observations may be gleaned from the data presented in tables 4–7, some of which may only be of theoretical importance.

Of considerable significance is the fact, disclosed by the data in these tables, that the proper pH is essential for bringing about suitable inversion at the lower temperatures used in this part of the investigation. For instance it is seen that even in those sirups which were heated for two hours at 175° F., there was little inversion except when the pH values were approximately 2.5 or below. Reasonably complete inversion was not obtained until the pH was reduced to about 2.1 or below. Whereas the optimum pH was about 2.0–2.6 for the boiled sirups, best results were obtained in the sirups inverted at 175° F. and 190° F. when the pH was adjusted to approximately 1.65–1.75.

It was suggested in the discussion of the boiled sirups that the apparent optimum hydrogen ion concentration in each series could only be considered as the optimum of the conditions represented. It is logical to believe that this is also true of the sirups prepared at the lower temperatures. In 15 of the 16 series of sirups inverted at 175° F. or 190° F., the highest TSI values were obtained in the batches of sirups having the lowest pH values. From this observation it seems that the present investigation could profitably be extended to determine more definitely the optimum hydrogen ion concentration for sirups to be inverted at temperatures below boiling.

On the basis of the results shown in tables 1, 4, 5, 6, and 7, there is a definite relationship among three factors, namely, temperature of heating, time of heating, and hydrogen ion concentration. It appears that when any one of these is increased, either or both of the others may be decreased.

The levulose content of the sirups prepared at 190° F. is seen to be lower than the dextrose content. This is especially pronounced in the batches with high pH, where the total reducing sugar content is quite low. This dextrose-levulose relationship is reversed in the sirups heated at 175° F. (see tables 6 and 7). The true explanation of this is not known, although Jordan's (3) statement that levulose will begin to caramelize if the solution is not cooled "immediately after the reaction has finished" suggests a lower heat stability of levulose as compared with dextrose. This may appear to be a reasonable explanation for the composition of the sirups inverted at 190° F., but it does not adequately account for the composition of sirups containing more levulose than dextrose, a condition which prevailed in nearly all batches heated only to 175° F.

By way of summarizing the practical results of the experiments reported in Tables 4 to 7 it may be said that either 190° F. or 175° F. produced satisfactory invert sirups, provided the length of the heating period and the pH were controlled properly. It has also been demonstrated that phosphoric acid was as good a catalyst as the more expensive tartaric acid.

SUMMARY AND CONCLUSIONS

The commercial "inversion" of sucrose by heating its water solution in the presence of acid seldom produces a sirup identical in composition with that which theoretically should be obtained.

Invert sirups prepared by boiling and by heating at 190° F. contained more dextrose than levulose. Sirups prepared by heating at 175° F. contained more levulose than dextrose.

On the basis of data obtained in the present investigation, it was found that the optimum acidity for inverting sucrose at boiling temperature was represented by a pH range of 2.0 to 2.6. With the lower heating temperatures (175° F. and 190° F.), pH values of approximately 1.65 to 1.75 were necessary to obtain satisfactory inversion.

Preliminary studies indicate that any of the following acids could be used for making invert sirup: tartaric, citric, lactic, hydrochloric, sulfuric, phosphoric.

There was a slight improvement in the inversion process as the water content of the uninverted sirup was increased from 27.5 per cent to 35.1 per cent.

Although some discrepancy was noted in the results secured with different boiling periods, the 30-minute period seemed to represent the best average.

Three factors influencing the inversion of sucrose appear to be positively interrelated. These are temperature of heating, time of heating, and hydrogen ion concentration. When any one or two of these is increased the other one or two may be decreased.

Invert sirup of good quality was prepared at 175° F. and 190° F. by holding the sirup at these temperatures for 45 to 90 minutes. The exact temperature and time were found to be less critical factors than pH.

Sirup prepared with phosphoric acid was found to be as good, on a TSI basis, as sirup prepared with tartaric acid.

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STUDIES OF THE CARBOHYDRATE METABOLISM OF MAMMARY GLAND TISSUE IN VITRO. II. THE METABOLISM OF CITRIC ACID AND B-HYDROXYBUTYRIC ACID IN TISSUE SLICES[1]

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While it has long been known that normal milk contains relatively large amounts of citric acid, no reference has been found as to its source. Analysis of simultaneously drawn arterial and mammary vein bloods failed to reveal any significant difference in the values for this substance indicating very little or no uptake of citric acid by the mammary gland from the blood.

To account for all of the citric acid found in milk by uptake from the blood would practically exhaust the latter of this substance in its course through the gland since it is several hundred times as concentrated in the milk as in blood. This fact suggests that citric acid is synthesized in the mammary gland and gives rise to speculation as to its blood precursors and the mode of synthesis.

Shaw and Knodt (4) working with intact cows and Shaw and Petersen (5) using the perfusion technique have demonstrated that the active mammary gland uses large quantities of β-hydroxybutyric acid. The fate of this substance in the gland, however, is only speculative. The possibility of its serving as a precursor of citric acid is suggested.

The investigation was undertaken in an attempt to ascertain whether or not citric acid is synthesized by the mammary gland and if so to determine the probable blood precursors as well as to endeavor to ascertain the probable fate of β-hydroxybutyric acid in milk formation.

EXPERIMENTAL

Because of the success experienced in a study of lactose formation by the use of tissue slices (2), this method of attack was suggested. The slices were prepared and incubated as described by Knodt and Petersen (2). In the study of citric acid formation a series of six experiments were run with each of the following additions: 1 per cent glucose, 0.5 per cent glucose and 0.5 per cent lactic acid, 1 per cent lactic acid, 1 per cent citric acid, 1 per cent pyruvic acid, 1 per cent maltose and 1 per cent glycogen. All experiments were run in duplicate and controls without any additions were also run with

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each series. The percentage of citric acid is expressed on the basis of tissue weight. Citric acid was determined by the methed of Pucher *et al.* (3).

The study of β-hydroxybutyric acid metabolism was made in another series of ten experiments in each of which in addition to the controls, duplicates with additions of 50, 100, and 200 mg. β-hydroxybutyric to 25 gm. tissue were incubated for 6 hours at 37.5° C. with oxygen aeration every 2 hours. In addition to citric acid determinations, analysis for β-hydroxybutyric acid, acetoacetic acid and acetone were made according to the method of Barnes and Wick (1). The acetoacetic acid and acetone were determined as acetone.

RESULTS

The results for incubation of 6 hours duration of controls and samples to which were added 1 per cent glucose, 0.5 per cent glucose and 0.5 per cent lactic acid, 1 per cent lactic acid, 1 per cent citric acid, 1 per cent pyruvic acid, 1 per cent maltose and 1 per cent glycogen are presented in table 1. It will be noted that in each experiment there is a significant increase in citric acid during incubation in the controls to which no additions were made. On the average, this increase was 9.19 mg. per cent from 17.53 to 26.72 mg. per cent.

Greater increases in citric acid were observed in every experiment where additions were made with the exception of those in which large amounts of citric acid were added. In the latter incubation there was a significant decrease in citric acid.

Results of the 10 experiments in which 50, 100, and 200 mg. β-hydroxybutyric acid were added are presented in the tables 2 and 3. In table 1 it will be seen that in all experiments a significant decrease in β-hydroxybutyric acid resulted and significant amounts of acetone and acetoacetic acid developed. No acetone bodies could be detected in the tissue without the addition of the β-hydroxybutyric acid either before or after incubation. It will also be noted that the use of β-hydroxybutyric acid increased with increments in the amounts added. The losses were 17.86, 33.09 and 92.4 mg., respectively for 50, 100, and 200 mg. additions of β-hydroxybutyric acid.

The amounts of acetone and acetoacetic acid formed during the incubation increased with increments in added β-hydroxybutyric acid. The average values being 5.29, 6.82 and 9.50, respectively, for 50, 100, and 200 mg. additions.

The effect of β-hydroxybutyric acid additions to incubated tissue slices upon citric acid formation is presented in table 3. Only slight increases in citric acid above those for the incubated controls are noted. The average values are 9.56, 10.57, 10.78 and 11.08 mg. respectively for the control and those to which, 50, 100 and 200 mg. additions of β-hydroxybutyric acid were made.

TABLE 1

The metabolism of citric acid during incubation of mammary gland tissue slices*

Experiment number	Before incubation		After incubation							
	Control	Control & 1% citric acid	Control	Glucose	0.5% glucose & 0.5% lactic acid	1% lactic acid	1% citric acid	1% pyruvic acid	1% maltose	1% glycogen
1	16.98	1011.8	25.95	47.80	35.46	34.57	798.0	40.93	31.41	29.42
2	17.28	1020.8	24.85	37.88	32.15	28.62	834.2	30.76	27.52	29.73
3	14.27	1021.8	27.92	48.05	51.26	30.20	885.5	34.47	35.21	32.17
4	23.16	1038.0	27.51	45.33	37.25	32.27	882.4	33.29	29.30	30.54
5	15.29	1016.0	32.49	50.21	38.94	34.76	860.7	41.62	37.21	43.85
6	18.20	1037.1	21.60	34.84	32.47	26.98	829.1	31.94	26.14	30.30
Average	17.53	1024.2	26.72	44.02	37.92	31.23	848.3	35.50	31.13	32.67

* Expressed as mg.% present in tissue.

TABLE 2

Utilization of β-hydroxybutyric acid by mammary gland tissue slices

Mg. β-hydroxybutyric acid added	1	2	3	4	5	6	7	8	9	10	Avg.
Mg. β-hydroxybutyric acid used by 25 gm. of tissue slices in 6 hrs.											
50	31.80	42.25	27.41	27.85	38.06	36.53	33.80	30.60	24.68	28.84	32.14
100	64.26	69.95	61.97	63.80	73.42	69.73	70.15	65.45	62.65	67.73	66.91
200	107.8	139.6	98.90	94.40	116.7	107.5	105.0	112.0	96.20	108.2	108.6
Mg. acetone and acetoacetic acid formed (as acetone)											
50	6.10	8.25	5.41	2.01	4.55	4.29	5.20	6.27	4.75	6.05	5.29
100	7.00	9.15	6.77	5.27	7.46	6.28	6.75	6.80	5.80	6.94	6.82
200	8.80	10.20	8.35	8.33	10.08	8.95	8.21	8.54	7.23	7.70	9.50

DISCUSSION OF RESULTS

It is significant that active mammary tissue slices incubated without any additions form citric acid. In all experiments, this increase was observed which amounted to 52.4 per cent. Since some time elapsed between slaughter and determination of citric acid in the tissue, it is reasonable to assume some formation had taken place in the interim. The citric acid formation under these conditions must be from materials present in the tissue presumably from the glycogen and other carbohydrates. This observation establishes the fact that the mammary gland can synthesize citric acid.

The increments of citric acid after incubation, over the controls following the addition of glucose, lactic acid, pyruvic acid, maltose and glycogen indicate that these substances can serve as precursors for citric acid formation in the mammary gland. The possibility, however, remains that these substances acted indirectly to increase the citric acid by stimulating other metabolic processes which in turn increase citric acid formation. It may be significant that the largest increases in citric acid are observed where glucose was added either alone or in combination with lactic acid. The significant average decrease of 175.9 mg. on incubation of tissue slices containing an average of 1024.2 mg. per cent citric acid indicates that the reaction can become reversed when the concentration becomes too great.

TABLE 3

The effect of added β-hydroxybutyric acid upon citric acid metabolism

Experiments	Citric acid present (expressed as mg. present)				
	Control before incubation	After incubation β-hydroxybutyric acid added			
		Control	50 mg.	100 mg.	200 mg.
1	4.61	7.71	9.45	9.59	9.85
2	4.41	7.40	9.54	9.51	9.92
3	5.54	8.81	10.26	10.48	10.88
4	7.97	10.79	11.26	11.63	12.35
5	7.23	10.03	12.12	12.32	12.54
6	10.12	12.84	13.54	13.98	13.98
7	6.53	8.91	9.48	9.61	9.97
8	6.24	8.68	8.82	8.98	9.32
9	7.79	9.71	10.01	10.40	10.85
10	8.57	10.75	11.19	11.33	11.30
Average	6.90	9.56	10.57	10.78	11.08

The addition of β-hydroxybutyric acid to incubated tissue slices confirms the observations of Shaw and Knodt (4), and Shaw and Petersen (5) in that the use of this substance by the mammary gland increases with its concentration. The observed increments in acetone and acetoacetic acid formation with increases in β-hydroxybutyric acid additions indicates that part of the latter is converted into these substances. Assuming a given amount of β-hydroxybutyric acid is converted into an equal amount of acetone and acetoacetic acid approximately 1/3, 1/5, and 1/10 of the β-hydroxybutyric acid used during incubation can be accounted for by this conversion respectively when 50, 100 and 200 mg. were added.

While increases in citric acid formation was observed with each increment of β-hydroxybutyric acid except in two instances out of the 30 observations the differences are so small as to be of questionable significance. At least in no instance was the citric acid formation comparable to that observed when glucose, lactic acid, pyruvic acid, maltose or glycogen were added.

SUMMARY AND CONCLUSIONS

A series of six experiments is reported on the formation of citric acid in incubated mammary tissue slices with and without the addition of glucose, glucose plus lactic acid, lactic acid, pyruvic acid, citric acid, maltose and glycogen. Ten other experiments on the fate of β-hydroxybutyric acid in mammary tissue slices is also reported.

It is shown that the mammary gland can synthesize citric acid from substances in the tissue.

Addition to the slices of glucose, lactic acid, pyruvic acid, maltose or glycogen increased citric acid formation indicating that these substances may serve as precursors.

When large amounts of citric acid were added its degradation was observed upon incubation.

Increments in added β-hydroxybutyric acid is used in increasing amounts by the incubated tissue slices.

Part of the added β-hydroxybutyric acid is converted into acetone and acetoacetic acid. The amount converted decreases with increased concentration and use.

Conversion of β-hydroxybutyric acid into citric acid by the mammary gland is questionable.

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STUDIES OF THE CARBOHYDRATE METABOLISM OF MAMMARY GLAND TISSUE IN VITRO. III. GLYCOGEN AS AN INTERMEDIARY IN THE FORMATION OF LACTOSE[1]

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Although the mammary gland has been shown to take up the carbohydrate portion of glycoproteins (17), to use lactic acid under certain conditions (6, 15) and to form lactose from glucose, maltose and glycogen in *in vitro* experiments (10, 11), blood glucose is considered to be the chief precursor of milk sugar (2, 7, 8, 9, 13, 19). The reduced milk secretion and lactose concentration following insulin administration (3, 5, 14) has been attributed to the resulting hypoglycemia suggesting that glucose is directly converted into the milk sugar in the mammary gland.

Reports by Petersen *et al.* (15) that lactating glands contain relatively large amounts, and non-active glands small amounts of glycogen raises the question of the rôle played by this substance in lactose synthesis. Demonstrations by Knodt and Petersen (12) that glycogen can be converted into lactose, suggest the possibility of the various blood precursors being first converted into glycogen but does not prove the point as the glycogen might serve merely as a carbohydrate reservoir to be drawn upon in case of inadequate blood precursors.

With this postulation the lowered lactose concentration following insulin administration would be accounted for by the blocking of glycogen degradation rather than by the resulting hypoglycemia.

An obvious test of the validity of this hypothesis would be maintaining the normal or increasing the blood glucose level with the administration of insulin, and noting the effect upon the lactose concentration in the milk and glycogen concentration in the tissue. If the lactose concentration should become less and the glycogen stores increase with insulin administration in the presence of adequate amounts of glucose such would support the hypothesis.

Since it would be difficult to maintain the above stipulated conditions in the intact animal and since normal milk secretion can be obtained in the perfused isolated bovine gland, where these conditions can easily be met, perfusion became the method of choice in this study.

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EXPERIMENTAL

A total of 17 perfusions were performed. In a series of 10 perfusions the additions of glucose were of the magnitude calculated to maintain normal blood levels. One half of each gland served as control while to the other half 160 units of insulin were added. In a series of 7 perfusions, large excesses of glucose were added, to produce a state of hyperglycemia, and from 120 to 160 units of insulin.

The perfusions were performed with a modified apparatus similar to that described by Petersen *et al.* (16) on one half of apparently normal lactating udders obtained from local abattoirs. Immediately after the perfusion was started, the glands were emptied of milk by injection of 10 units of oxytocin. Insulin was then added to the blood. In all experiments the glands were emptied by the use of oxytocin after 1 hour of perfusion and then hourly or every 2 hours up to 4 or 5 hours. The amounts of milk obtained at each milking was ascertained and analyzed for lactose.

Lactose was determined on each sample of milk according to the method of Bierman and Doan (1). Blood glucose was determined according to the methods of Schaeffer and Somogyi (18) and tissue glycogen according to Good, Kramer and Somogyi (4). Chlorazol fast pink was used in the blood as an anticoagulant. The insulin used was "Regular Insulin" produced by Eli Lilly and Company.

RESULTS

In table 1 are presented the results of perfusing with approximately normal amounts of blood glucose. In the first 5 experiments (Group I) insulin was not administered. To each of the second group of five experiments (Group II) 160 units of insulin was added. While there is a small progressive drop in lactose content with the successive milkings in the experiments where insulin was not administered in the experiments where insulin was added the drop is precipitous. In the former the original milk contained 3.77 per cent and ended after 5 hours of perfusion with 2.84 per cent of lactose while in the insulinized glands the respective values were 3.62 and 0.65 per cent.

The glycogen contents of the glands of the two groups were essentially the same at the beginning of the experiment, averaging 22.52 and 23.32 mg. per cent, respectively, for Group I and Group II. Although perfusion for 5 hours increased the glycogen content of the glands in both groups the average final values were approximately 2.5 times as high in the glands that were insulinized as in the controls. The blood glucose levels at the end of five hours of perfusion varied in both groups to such an extent as to preclude conclusions as to trends.

There is great variability within each group as to the quantities of milk obtained at the different milkings. No significant difference between the two

TABLE 1

The effect of insulin upon lactose and glycogen formation in the perfused mammary gland with normal blood glucose

Perfusion number	Milks										Final blood glucose	Glucose added	Insulin added
	Original		0–1 hour		1–3 hours		3–5 hours		Tissue				
	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose	Glycogen original	Final			
	Group I. Non-insulinized glands												
	ml.	*%*	*ml.*	*%*	*ml.*	*%*	*ml.*	*%*	*mg.%*	*mg.%*	*mg.%*	*gm.*	*units*
197	1400	3.70	500	3.38	330	3.09	290	2.99	26.05	62.50	22.50	5	0
198	1400	4.02	760	4.11	370	3.85	280	3.60	19.75	42.09	68.55	10	0
201	4460	3.58	460	3.67	270	3.08	220	2.90	23.17	65.43	61.60	10	0
202	1560	3.82	530	3.43	310	3.27	265	2.77	22.50	69.20	47.33	10	0
204	880	3.72	980	3.20	340	2.85	290	1.94	21.13	28.05	41.30	10	0
Ave.	1940	3.77	646	3.56	324	3.23	269	2.84	22.52	53.45	48.26	9	
	Group II. Insulinized glands												
196	220	3.82	820	3.19	350	1.35	210	0.46	20.35	116.2	55.00	5	160
199	1540	3.70	850	3.74	195	1.86	45	0.42	21.44	145.7	48.35	10	160
200	1400	3.54	240	3.44	360	1.69	120	0.90	18.58	187.8	43.07	10	160
203	1090	3.47	240	2.65	380	1.58	200	0.67	25.00	92.2	57.30	10	160
205	760	3.58	1340	2.04	650	1.46	120	0.79	31.25	127.0	106.8	10	160
Ave.	1002	3.62	698	3.01	387	1.59	139	0.65	23.32	133.8	62.1	9	160

TABLE 2

The effect of insulin upon lactose and glycogen formation in perfused bovine mammary glands with large amounts of added glucose

Perfusion number	Milks										Final tissue glycogen	Glucose added	Insulin added
	Original		1 hour		2 hours		4 hours		5 hours				
	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose			
	ml.	*%*	*ml.*	*%*	*ml.*	*%*	*ml.*	*%*	*ml.*	*%*	*mg.%*	*gm.*	*units*
189	520	4.32	300	3.16	260	2.77	125	0.75	80	0.56	372.0	37.8	130
190	760	4.06	295	2.18	740	2.18	560	0.92		...	158.6	37.8	120
191	1090	4.51	510	3.08	290	1.53	170	0.85	.	...	259.7	100.0	160
192	1260	3.94	320	3.07	250	1.95	210	1.04			288.0	50.0	160
193	1050	3.96	560	3.36	320	2.07	300	0.73		...	214.2	50.0	160
194	850	3.77	*		620	2.38	230	1.31	60	0.49	.	60.0	160
195	1040	4.12	*		640	1.75	280	0.63		..	...	60.0	160
Ave.	939	4.10	397	2.97	446	2.09	268	0.89	70	0.53	258.5	56.5	...

* Not milked until 2 hours after beginning of perfusion. Final blood glucose determined on perfusions 194 and 195 were 272.4 and 341.0 mg. per cent respectively.

groups is observed until the last milking—after five hours of perfusion. Here the average quantity of 139 ml. of the insulinized group as compared to 269 ml. for the controls is significantly lower.

The results of seven experiments, all insulinized, to which large quantities of glucose were added, are presented in table 2. In two cases only was the glucose level in the blood determined revealing 272.4 and 341.0 mg. concentration for perfusions 194 and 195, respectively. From the observations made when 5 and 10 gm. of glucose were added it is certain that in all seven experiments a marked hyperglycemia was maintained throughout the experiments.

Five of the experiments were discontinued at the end of four hours while others were conducted for a longer period of time. At that time the lactose concentration in the milk had dropped from an average of 4.1 per cent at the beginning to 0.89 per cent. For the two experiments continued for five hours the lactose dropped to 0.56 and 0.49 per cent.

The glycogen concentration in the gland tissue was determined only in the first five experiments. In these the average concentration was 258.5 mg. per cent as contrasted to an average of 113.8 mg. per cent in the five previously cited experiments in which insulin was administered and the blood glucose was maintained at normal levels. The difference is of greater significance than the data indicates because of the shorter duration of the perfusions with high blood glucose. Four of the five in the latter were perfused only four hours, while in the low blood glucose group all were perfused five hours. Significantly the gland perfused five hours had by far the greatest glycogen concentration—372.0 mg. per cent.

DISCUSSION OF RESULTS

It is clear from the data presented that under the conditions of these experiments insulin decreased lactose formation, which was observable in the milking one hour after its administration. Just when and to what extent lactose formation was inhibited following the administration of insulin cannot be ascertained from these data because of the indeterminable amounts of residual milk remaining in the ducts and alveoli following each attempted evacuation by the use of oxytocin. It is reasonable to suppose, however, that the residual milk was not quantitatively sufficient to account for a major portion of the lactose found in the milks following insulin administration and therefore the inhibitory action of the insulin developed more or less gradually.

It is also clear that glycogen contents of the insulinized glands is significantly higher than the controls when perfused with bloods with normal glucose levels. This increase in glycogen can be accounted for by either or both of two postulates—that insulin facilitated the conversion of glucose into glycogen or it merely blocks glycogen breakdown without affecting the rate of its formation. Unfortunately the data taken are not adequate to conclusively settle the question, but there is some indication that the insulin action is mainly that of blocking the breakdown of glycogen rather than accelerating its formation in the mammary gland.

Although weights were not taken and there was considerable variation in the sizes of glands used in the experiments, but since efforts were made to pair the glands used in Groups I and II it is fair to assume that the average weights were approximately the same for the two groups. From the data in table 1 it is calculated that 12.75 gm. more lactose was secreted in the milks obtained during the perfusion from the controls than from the insulinized glands. The insulinized glands contained 70.4 mg. per cent more tissue glycogen than the controls. To account for the equivalent of 12.74 mg. lactose in additional glycogen stores of 70.4 mg. per cent would require 18.2 kilos of gland tissue which exceeds by far the average weight of the glands used.

It is conservatively estimated that no gland used exceeded 10 kilos in weight and the average would be considerably less.

A second support for this postulate is found in the glucose concentration in the bloods at the termination of the experiments which shows no significant difference between the controls and the insulin-treated gland. If insulin should accelerate glycogen formation from glucose then the blood glucose levels should be lower in the experiments where insulin was used.

In table 2 it is seen that when the blood glucose is unusually high with insulin the glycogen deposition in the tissue becomes proportionately higher, an average of 258.5 mg. per cent for 5 experiments with a high of 372.0 mg. per cent for 1 experiment. However, Knodt and Petersen (12) have reported an average glycogen content of 231.7 mg. per cent with a high of 514 mg. per cent in a series of 20 similar experiments except that insulin was not administered.

With these facts as a basis it is postulated that lactose may be formed in the mammary gland from the degradation of glycogen. In all of the experiments reported herein there has been sufficient or an excess of glucose in the blood to produce milk with normal lactose content if that substance is converted directly into the milk sugar.

It is to be recognized that argument may be advanced that insulin interferes with other metabolic processes in the cell which prevents lactose formation. If such is the case it must be pointed out that such a metabolic disturbance must act preferentially on lactose formation, because, while there is evidence of decreased milk secretion from insulin administration, the depression of lactose synthesis is much greater.

The fact that the increased glycogen storage in the insulin experiments cannot account for the glucose spared by decreased lactose formation indicates a greater utilization of glucose for other purposes. While no attempt is made here to review the extensive literature on the action of insulin it may be stated that the indicated greater use of glucose is controversial as is the theory of insulin blocking the degradation of glycogen and the stimulating effect on glycogen formation.

SUMMARY AND CONCLUSIONS

The results of a series of 17 perfusion experiments are reported in which the effects of insulin upon lactose formation and tissue glycogen storage were studied. The effects of insulin administered in a series of 5 experiments were compared with the results in 5 experiments where insulin was not added but glucose added to maintain normal or subnormal blood levels. In 7 experiments the effect of large additions of glucose together with insulin was studied.

Administration of 120 to 160 units of insulin markedly reduced the percentage of lactose in the milks even though the bloods contained adequate amounts of glucose for normal lactose production.

The accumulation of tissue glycogen was greater in the insulinized glands than in the controls.

Evidence is presented to indicate that the increase in tissue glycogen was not due to glycogenic properties of insulin but more likely to its blocking of glycogenolysis.

It is postulated that lactose is formed from degradation of glycogen and the lowered lactose production in experiments where insulin was administered is accounted for by a blockage of glycogen breakdown.

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ASSOCIATION ANNOUNCEMENT

ANNUAL MEETING, AMES, IOWA, JUNE 18–20, 1946

IOWA INVITES YOU

To the Officers and Members of the American Dairy Science Association:

The Iowa State College joins with the Dairy organizations of Iowa in expressing great satisfaction that the American Dairy Science Association has decided to hold its 1946 annual meeting on our campus the eighteenth, nineteenth and twentieth of June.

As one of the great dairy states of America, we welcome you. We recall with pleasure your meeting at the College in 1930; and we are looking forward with even greater anticipation to your visit next June. We hope that the 1946 meeting of the Association will contribute materially to the success of the dairy industry of the nation.

Sincerely yours,
Charles E. Friley
President

FIRST CALL FOR PAPERS

Members who wish to give original papers at the annual meeting should send the titles to the Program Committee as promptly as convenient. All titles must reach the Program Committee before March 25, but early receipt of the titles is of real value in arranging the best program. All communications regarding general plans should be addressed to the chairman of the General Program Committee, but all titles of papers should be sent directly to the chairman of the Program Committee for the section before which the paper should be presented.

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STUDIES ON KETOSIS IN DAIRY CATTLE. VII. THE EFFICACY OF B VITAMINS AND METHIONINE IN THE TREATMENT OF KETOSIS[1]

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Following the report by Carlström *et al.* (1) that ketosis in cattle could be cured by thiamine administration, a number of articles have appeared in the literature supporting the claim and thiamine has been given wide usage in the treatment of ketosis in ruminants. Unfortunately little or no chemical data have been reported to confirm the diagnosis of ketosis or the reported recovery of the animals following thiamine therapy.

In the present investigation a study was made of the therapeutic value of thiamine and other B vitamins in the treatment of ketosis in dairy cows. Observations were also made on the effectiveness of methionine in the treatment of this condition. The B vitamins and methionine were furnished through the courtesy of Merck and Company.

EXPERIMENTAL

Only cows with uncomplicated ketosis were used in these studies. Diagnosis was made on the basis of the recognized signs and symptoms of ketosis in cows supported by data on blood glucose and acetone bodies. During the course of treatment every effort was made to maintain environmental conditions as they were when the condition developed. In particular no changes were made in feeds or in feeding practices.

Seven cows with ketosis received thiamine alone or in combination with other B vitamins. The B vitamins were supplemented with glucose administration in two cases. Two cows with ketosis received methionine.

Cow D.E. 73 (table 1) received 100 mg. thiamine hydrochloride per os daily for seven days, followed by a mixture of B vitamins daily for 10 days. This included 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, and 1200 mg. calcium pantothenate. During this period there was only slight improvement, the blood glucose

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increasing from 19.0 to 26.6 mg. per cent and the blood acetone bodies decreasing from 27.23 to 15.33 mg. per cent. This developed into a chronic case in which the blood glucose and acetone bodies remained abnormal for some time after the symptoms of ketosis had disappeared.

Cow D.E. 74 (table 2) had received glucose injections before this study was initiated and appeared to have recovered. With the recurrence of symptoms 22 days after parturition, B vitamins were administered per os for a period of 17 days. The dosage was the same as in the previous case. There was some improvement in the blood picture and in the general appearance of the animal during this period. Milk production increased for a few days and then fell off again.

TABLE 1

The efficacy of B vitamins in the treatment of ketosis (D.E. 73)

Days	Blood glucose	Blood acetone bodies (as acetone)	Milk production	Treatment	Comments
	mg.%	*mg.%*	*lbs.*		
1					Calved
8	19.0	27.23	49.5	100 mg. thiamine per os daily	Lethargy, inappetence
11	24.0	12.65	53.0	"	
13	34.0	14.00	56.7	"	
15	18.6	14.90	61.5	B vitamins* daily per os	Appetite improved
17	20.8	16.35	62.3	"	
19		18.18	64.0	"	
21		25.02	64.1	"	
23	42.6	26.07	65.8	"	
25	24.6	19.46	67.0	"	
27	26.6	15.33	68.4	Discontinued treatment	
31	28.8	14.04	67.0		
34	26.8	10.40	67.9		
37	26.4	19.23	69.6		Slight inappetence only
39	20.0	16.99	69.8		Appeared normal
42	25.2	12.00	72.1		"
45	29.0	12.81	72.6		"
48	34.6	13.43	77.1		"
61	41.3		68.9		"

* 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, 1200 mg. calcium pantothenate.

Cow D.E. 75 (table 3), representing a mild case of ketosis, received 100 mg. of thiamine hydrochloride per os daily for seven days followed by B vitamins for 10 days. During the first seven days of treatment the condition of the animal became worse, as shown by the blood picture and the symptoms. After seven days of treatment the animal recovered rapidly.

Cow D.E. 76 had a severe case of ketosis as shown by both the blood picture and the symptoms. As indicated in table 4, B vitamins were administered per os for a period of five days. By the sixth day the condition

TABLE 2

The efficacy of B vitamins in the treatment of ketosis (D.E. 74)

Days	Blood glucose	Blood acetone bodies (as acetone)	Milk production	Treatment	Comments
	mg.%	*mg.%*	*lbs.*		
1	......	......			Calved
6	......	.	69.4	250 gm. glucose intravenously	Lethargy, inappetence
8	..	. .	74.2	"	"
18	.		79.0		Improved
22	21.4	14.16	72.6	B vitamins* per os daily	Lethargy, inappetence
24	18.0	17.05	71.3	"	Same
26	20.6	12.87	74.0	"	"
29	23.4	15.07	79.9	"	"
32	30.4	10.81	90.4	"	Appetite improved
36	32.8	5.84	94.4	"	
39	29.4	12.38	82.5	"	Slight lethargy
41	30.4	12.13	79.9	Discontinued treatment	"
45	32.6	8.31	78.1		"

* 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, 1200 mg. calcium pantothenate.

of the cow was so poor that glucose was administered intravenously, after which recovery was fairly rapid.

Cow D.E. 77 (table 5) exhibited severe ketosis the day following parturition. B vitamins were administered in the dosage indicated in table 5 for a period of four days. The condition became so severe that glucose was

TABLE 3

The efficacy of B vitamins in the treatment of ketosis (D.E. 75)

Days	Blood glucose	Blood acetone bodies (as acetone)	Milk production	Treatment	Comments
	mg.%	*mg.%*	*lbs.*		
1					Cow calved
4	35.6	18.98		100 mg. thiamine hydrochloride per os daily	Slight lethargy
6	30.0	23.54		"	
8	18.8	28.44		"	
10	23.6	30.25	31.7	B vitamins* per os daily	Lethargy, inappetence
12	36.8	8.38	39.0	"	Appetite improved
18	55.6	7.40	46.2	"	
20	47.4	6.99	48.2	Discontinued treatment	Normal appearance
25	43.6	3.76	47.7		
30	41.4	3.20	47.8		
35	41.6	3.24	48.0		

* 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, 1200 mg. calcium pantothenate.

injected intravenously on the sixth and eighth days after parturition. Some slight response was noted. B vitamins were then administered per os for a period of 10 days supplemented by feeding three pounds of glucose daily mixed with the grain. After seven days of such treatment, glucose was again injected intravenously because of the acute condition of the animal. This cow was consuming from 18 to 20 pounds of concentrate and approximately 20 pounds of mixed hay throughout most of this period but became quite emaciated. When it became apparent that the B vitamins were ineffective, the cow was used for other studies. This cow eventually recovered and produced over 700 pounds of butterfat.

TABLE 4

The efficacy of B vitamins in the treatment of ketosis (D.E. 76)

Days	Blood glucose	Blood acetone bodies (as acetone)	Milk production	Treatment	Comments
	mg.%	*mg.%*	*lbs.*		
1					Calved
9			57.4		
10	16.0	30.50	51.0	B vitamins* per os daily	Lethargy, incoordination
11	19.6	36.05	52.2	"	No change
12	22.4	38.90	50.0	"	"
13	14.4	40.54	51.5	"	"
14	18.6	31.52	52.7	"	"
15	18.0	38.60	52.3	280 gm. glucose intravenously	Inappetence, paresis of neck, marked incoordination
16	29.2		60.5	No further treatment	Definite improvement
17	26.7	29.86	54.6		
19	26.8	24.65	54.3		
20	32.6		57.0		
21	36.7	16.88	59.5		Normal appearance
24	28.6	18.00	58.5		
26	41.2		61.5		
29	37.3		64.0		
31	42.7		64.5		
35	33.4		61.7		
39	35.4		64.8		Normal appearance

* 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, 1200 mg. calcium pantothenate.

D.E. 198 (table 6) exhibited the first symptoms of ketosis the tenth day after parturition and gradually became worse. The cow was not treated and the condition became acute by the thirtieth day. Relatively massive doses of B vitamins were used in this case. For the first four days one gram of thiamine hydrochloride per day was injected intravenously. By the fifth day it was necessary to administer glucose to save the cow. Two intravenous injections of 250 grams each were made in three days. For the next three days the following vitamins were administered daily by the intravenous route: thiamine hydrochloride, 1 gm.; pyridoxine, 1 gm.; nicotinamide, 5 gm.; and calcium pantothenate, 5 gm. The condition of the cow again became so acute that glucose was again administered intravenously. The blood picture showed some improvement during this period but it was apparent that the cow was on the verge of collapse. The sharp rise in blood sugar, to a level considerably above normal, which occurred after the cow was allowed to graze has frequently been observed in cows which recover quickly from ketosis. Such a rise was also noted in cow D.E. 75, table 3, and in a number of other cases not reported. The return to a normal level of glucose within a day or so from the abnormally high level observed in these two cases has also been noted before.

TABLE 5

The efficacy of B vitamins in the treatment of ketosis (D.E. 77)

Days	Blood glucose	Blood acetone bodies (as acetone)	Milk production	Treatment	Comments
	mg.%	*mg.%*	*lbs.*		
1	68.0	2.83			Calved
2	31.4	7.97		B vitamins* per os daily	Incoordination
3	35.8	5.99		"	Slight improvement
4	21.2	13.26	41.9	"	No change
5	17.8	21.42	46.1	"	Marked incoordination
6	12.4	21.92	48.0	290 gm. glucose intravenously	Marked incoordination, inappetence
7			48.4	No treatment	Marked incoordination
8	23.4	21.60	49.5	250 gm. glucose intravenously	Marked incoordination
9			50.9	No treatment	Slight incoordination
10	21.2	29.64	49.0	No treatment	Slight incoordination
11	24.6	36.15	50.7	B vitamins* + 3 lbs. glucose per os	Slight lethargy
12	24.7	23.00	56.5	"	"
13			58.8	"	"
14			56.4	"	"
15	16.8		58.8	"	Lethargy
16			57.7	"	"
17	21.8	21.82	55.1	B vitamins* + 3 lbs. glucose per os + 250 gm. glucose intravenously	Paresis of neck, marked incoordination, weak
18			56.8	B vitamins* + 3 lbs. glucose per os	Slight improvement
19			58.2	"	Incoordination
20	17.0	19.41	57.8	"	"

* 100 mg. thiamine hydrochloride, 60 mg. riboflavin, 60 mg. pyridoxine, 1200 mg. nicotinic acid, 1200 mg. calcium panthothenate.

Probably the most conclusive study on the use of B vitamins for ketosis in cows was that conducted on cow 230 (fig. 1). This cow refused to eat grain and ate but very little hay. Sufficient response was obtained by the initial injection of glucose and calcium gluconate to indicate that the cow would have recovered rather quickly had glucose therapy been continued. The blood calcium was 9.62 mg. per cent, showing that the condition was not complicated with milk fever. Blood hemoglobin was 11.48 per cent. Massive doses of B vitamins were given in capsules at daily intervals for 12 days. Intravenous administration of B vitamins was also made on each day during the first eight days of treatment. The B vitamins included thiamine hydrochloride, nicotinic acid, calcium pantothenate, riboflavin, pyridoxine, para aminobenzoic acid, inositol, biotin, and choline. The dosage is shown in figure 1. It will be noted from the data in figure 1 on blood glucose, blood acetone bodies, and milk production that the massive doses of B vitamins were completely ineffective in alleviating the condition of the cow. There were no complications, the cow recovering several days later after a change of treatment.

TABLE 6

The efficacy of B vitamins in the treatment of ketosis (D.E. 198)

Days	Blood glucose	Blood acetone bodies (as acetone)	Treatment	Comments
	mg.%	*mg.%*		
1		.		Calved
10		...		Inappetence
30	26.9	48.38	1 gm. thiamine intravenously	Paresis of neck, scoliosis, general stiffness, eating hay only
31	23.3	52.06	"	"
32	25.5	48.76	"	"
33	23.3	48.28	"	"
34	22.0	.	250 gm. glucose intravenously	"
35			No treatment	Some improvement in general condition
37	23.1	54.50	250 gm. glucose intravenously	Paresis of neck, scoliosis, general stiffness
38	32.5	23.66	B vitamins* intravenously	Slight improvement
39 A.M.	25.6	28.92	"	No change
P.M.	34.3	30.65	0.5 gm. riboflavin intravenously	"
40	34.4	23.66	250 gm. glucose + B vitamins† intravenously	Paresis of neck, scoliosis, general stiffness
41	44.5	14.44		Slight improvement
42	56.3	5.12	On pasture	Marked improvement

* 1 gm. thiamine hydrochloride, 1 gm. pyridoxine, 5 gm. nicotinamide, 5 gm. calcium pantothenate.

† 0.5 gm. riboflavin added to above vitamins.

In table 7 data are presented on two cases which received *dl*-methionine for several days. Cow D.E. 263 received 12.5 gm. of methionine intrave-

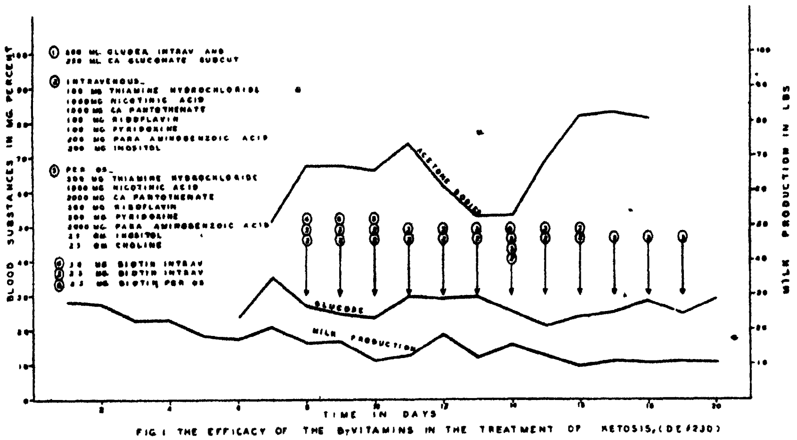

FIG. 1. The efficacy of the B vitamins in the treatment of ketosis (D.E. 230).

nously for five days, during which time the condition of the cow became worse. Improvement was rapid following glucose administration. Cow D.E. 265 received daily administrations of 12.5 gm. of methionine intravenously and 12.5 gm. orally for a period of eight days. The condition of the

TABLE 7

The efficacy of methionine in the treatment of ketosis

Cow	Days	Blood glucose	Blood acetone bodies (as acetone)	Treatment	Comments
		mg.%	*mg.%*		
D.E. 263	1	28.3	49.36	12.5 gm. methionine intravenously	Inappetence, lethargy
	2	25.5	55.11	"	"
	3	27.7	46.67	"	"
	4	25.0	47.39	"	"
	5	20.4		"	"
	6	24.9		250 gm. glucose intravenously	No improvement to date
	7	35.2		..	Improved appetite
	8	43.6	11.58		Alert, improved appetite
D.E. 265	1	29.2		12.5 gm. methionine intravenously + 12.5 gm. per os	Inappetence, incoordination
	2	29.0	24.61	"	"
	3	16.4	26.09	"	"
	4	16.8		"	"
	5	13.5		"	"
	6	19.5	37.24	"	"
	7	21.3	39.94	"	"
	8			"	"
	9	15.1	36.23		"

cow was acute by the ninth day and use of methionine was discontinued. Both cows recovered within a relatively short period of time after the intravenous administration of glucose.

DISCUSSION

In evaluating the results of this investigation it is important to bear in mind that the majority of cases of uncomplicated ketosis in cattle will recover without treatment, some rapidly and others only after a protracted period of time. D.E. 75 was the only cow which recovered rapidly following the administration of B vitamins. However, in this case the condition of the cow deteriorated rapidly during the first four days of treatment when thiamine hydrochloride was being administered, after which other B vitamins were added and recovery was prompt. In the other cases there was little or no apparent response. There was likewise no indication that glucose therapy was more effective when supplemented by thiamine and other B vitamins. Cow D.E. 77 received thiamine hydrochloride, riboflavin, pyridoxine, nicotinic acid, calcium pantothenate, and three pounds of glucose daily for a prolonged period of time without any apparent beneficial effect.

Blood chemistry data are presented on cows D.E. 73 and D.E. 77 over some period of time following cessation of treatment because these two cases are quite typical of a large number of cases in the field which appear to have recovered following treatment but which continue to exhibit hypoglycemia and ketonemia for a prolonged period. Similar observations were made in earlier reports (2, 3). Frequently such cases suffer a relapse which could have been avoided in the majority of cases by feeding some form of readily available carbohydrate for a few weeks after intravenous glucose administration was discontinued. It is entirely possible that many of the cases of ketosis which are reported to occur several weeks or months following parturition may be merely outward symptoms of a condition which has existed for some time, in which symptoms may or may not have been in evidence at an earlier date.

CONCLUSIONS

1. Thiamine hydrochloride when administered alone either orally or intravenously or in combination with nicotinic acid, calcium pantothenate, riboflavin, pyridoxine, para aminobenzoic acid, inositol, choline, and biotin, was ineffective in the treatment of ketosis in lactating cows. Likewise the efficacy of glucose did not appear to be enhanced by the administration of thiamine hydrochloride, riboflavin, pyridoxine, nicotinic acid, and calcium pantothenate.

2. Methionine was ineffective in the treatment of ketosis when administered either orally or intravenously.

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THE ESTIMATION OF THE WEIGHT OF BULLS FROM HEART GIRTH MEASUREMENTS

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Though significant relationships between heart girth measurements and weight of dairy cows (1, 5) and beef cattle (2, 3, 4, 6, 7) have been found, no evidence of this nature has been reported on mature dairy bulls. Wanderstock and Salisbury (7) concluded that the most significant single measure for estimating weight of beef cattle is heart girth measurement. If this method could be used as a practical means of estimating the weight of mature dairy bulls, it would be of great help when scales are not available.

The object of the analyses in this paper was to study the relationship of heart girth measurements and weight of mature dairy bulls, also, to test the accuracy of the estimation of the weight of a dairy bull from heart girth measurement.

SOURCE OF DATA

The bulls from which the data used in this study were obtained were 50 Holstein and Guernsey bulls in the New York Artificial Breeders' Cooperative, Inc., herd. All of these were mature bulls except for two Holstein bulls and one Guernsey bull of about two and one-half years of age. The bulls were in good breeding condition but carried no excess flesh. Except for seven experimental bulls, all were being fed and routinely used by the Cooperative at the time the measurements and weights were taken.

After the animal was weighed on a set of platform scales, it was placed squarely on all four feet with its head in the normal position. A cloth tape, about one inch wide and graduated in inches, was used to take the heart girth measurements. The measurement was taken by placing the tape around the animal at the point of smallest circumference just behind the forelegs. It was pulled snugly about the animal, tight enough to keep the hair down but not tight enough to indent the flesh.

ANALYSIS OF DATA

For purposes of analysis, the data on all bulls were used to calculate the correlation coefficients and regression. Then the data were separated according to breeds and calculations made. All calculations of correlation coefficients and regression lines and their significance were made and interpreted according to Snedecor (8).

RESULTS

The weights of the bulls ranged from 852 to 2351 pounds. The mean weight was 1659.88 ± 377.07 pounds and the mean heart girth measurement

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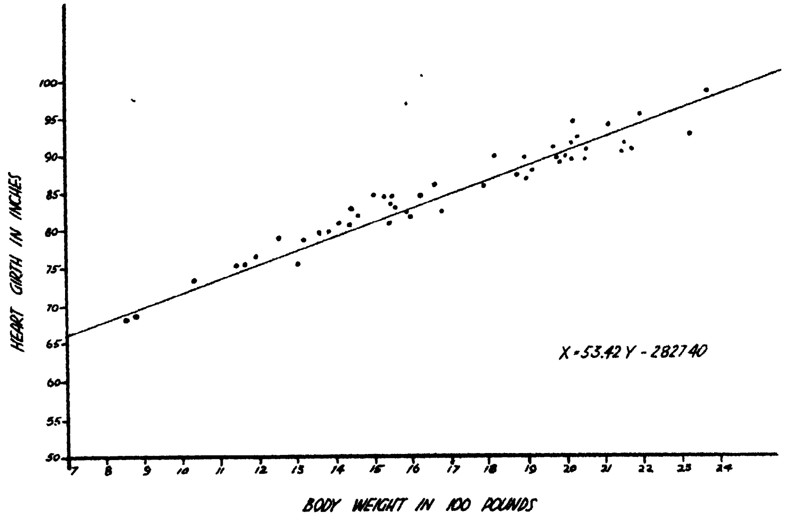
was 83.92 ± 6.89 inches. The correlation coefficient showing the relationship between the heart girth measurement and body weight of 50 Holstein and Guernsey bulls was 0.976. Correlation coefficients for 25 Holsteins and 25 Guernseys were 0.954 and 0.958 respectively. All correlation coefficients were highly significant mathematically.

A calculation of the regression of actual live weights on the heart girth measurement indicated a straight-line relationship existed. The equation for the line was $X = 53.42\ Y - 2827.40$, where X = the calculated estimate of the weight and Y = the measured heart girth. The standard error of the regression line was 83.8 pounds, or 5.05 per cent of the mean, a relatively low standard error for data of this type. The regression line for all bulls is given in the accompanying graph.



REGRESSION OF BODY WEIGHT ON HEART GIRTH MEASUREMENT OF DAIRY BULLS

Having the body weights and semen volume data on hand, the authors were interested in knowing whether or not there was a relationship between weight of a bull and semen volume. The average volume of semen was calculated from collections made from bulls between May 15 and August 15, 1945, considering all ejaculates. The correlation coefficient between body weight and semen volume was 0.2079, which was not significant mathematically.

SUMMARY

A study was made of the relationship between heart girth measurement and the body weight of 25 Holstein and 25 Guernsey bulls ranging in weight from 852 to 2351 pounds.

The coefficients of correlation between heart girth measurement and body weight were 0.976 for all of the 50 bulls, 0.954 for the Holstein bulls, and 0.958 for the Guernsey bulls; all were highly significant statistically.

A calculation of the regression of body weight on heart girth measurements indicated a straight line relationship. The error of estimate of body weight based on heart girth measurement was 83.8 pounds or 5.05 per cent. Estimates of the body weights of bulls were, therefore, very accurate.

It was also of interest to note that the correlation coefficient between body weight and semen volume was 0.2079, which was not mathematically significant.

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THE RATE OF AUTOXIDATION OF MILK FAT IN ATMOSPHERES OF DIFFERENT OXYGEN CONCENTRATION*

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Henderson and Young (1) determined the rate of autoxidation of oleic acid in atmospheres of 21 to 100 per cent oxygen and concluded that it varies as the square root of the oxygen concentration. Although oleic acid constitutes approximately 90 per cent of the unsaturated fat acids in milk fat, it is doubtful if Henderson and Young's conclusion is applicable to milk fat under conditions wherein lower oxygen concentrations are concerned. Lea, Moran, and Smith (2) noted the effect of low initial oxygen concentrations in containers of dried milk on the tendency of the fat to become tallowy and concluded that concentrations below approximately 3 per cent are satisfactory for use in a commercial pack. The results did not lend themselves to an estimation of the rate of oxidation.

Greenbank, Wright, Deysher, and Holm (3) determined the keeping quality of commercial samples of dried milks packed in atmospheres of different percentages of oxygen and stored at different temperatures. The conclusion with respect to the O_2 concentration that would be effective in greatly retarding deterioration was similar to that arrived at by Lea, Moran, and Smith, but the data obtained were not extensive enough to indicate the variations in rate of oxidation with variations in oxygen concentration.

EXPERIMENTAL

Autoxidation of milk fat. A certain degree of oxidation of a fat can occur before tallowy flavors are produced. This degree of oxidation varies with the type of fat and also with the temperature at which the oxidation occurs, becoming greater as the unsaturation of the fat increases and less as the temperature increases.

With milk fat the degree to which oxidation can occur before tallowy flavors are produced is small at relatively low temperatures of storage, hence the amount of available oxygen must be small if the development of tallowy flavor is to be prevented during long periods of storage. Since the products of decomposition of the initial oxidation products (peroxides) are responsible for the tallowy flavor and since the decomposition proceeds progessively with increases in time even at low temperatures, a value of interest in the

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* Data presented at the Technical Conference of the Dry Whole Milk and Ice Cream Mix Industry under the auspices of the Q.M.C. Subsistence Research and Development Laboratory, January 23–25, 1945, Chicago, Illinois.

packaging of milk fat or dried milk in an inert gas is the minimal amount of oxygen which when it reacts with milk fat will produce a tallowy flavor. This value for milk fat was determined as follows:

Samples of milk fat varying from 15 to 50 g. in weight were placed in ampoules and evacuated thoroughly at a temperature of 80° C. with continuous shaking. When all of the dissolved air had been removed in this manner, calculated amounts of oxygen were bled into each tube, a 3-ml. azotometer with mercury as the confining liquid being used as the measuring device. Nitrogen was then allowed to flow in until atmospheric pressure was attained, and the tubes were sealed. In this manner a series of ampoules containing oxygen concentrations of from 0.5 to 9.0 per cent by volume was prepared and samples of each series were stored at temperatures of 70°, 50°, and 30° C.

After prolonged storage only those samples of less than 0.80 per cent oxygen in the ampoules did not develop tallowy flavors. This concentration of oxygen is equivalent to approximately 0.009 ml. of oxygen per gram of fat. A concentration of 0.013 ml. per gram of fat (1.19 per cent by volume oxygen concentration) produced a distinctly oily and tallowy flavor after 16 weeks of storage at 55° C. Hence, to prevent the production of a tallowy flavor in butteroil through oxidative changes under severe conditions of storage, the amount of available oxygen should be less than 0.80 per cent of the volume of the fat (4).

To obtain an approximation of the variation in rate of autoxidation of milk fat with variations in the oxygen concentration of the atmosphere in which it is packed, experiments with pure milk fat were carried out in which the oxygen concentration of the atmosphere was varied from 1.70 to 100 per cent.

In the experiments with butteroil the autoxidation was carried out at 70° C. and the rate measured by determining the peroxide value at intervals in the reaction.

The extent to which the rate of autoxidation of a fat is dependent on the concentration of oxygen of the atmosphere with which it is in contact is indicated in figure 1, by the value for the rate of peroxide formation in pure milk fat with nitrogen-oxygen mixtures of different oxygen concentration. In figure 2 is shown the time required for the peroxides to reach a value of 5.0 in atmospheres of different concentrations of oxygen.

These values indicate that, comparatively, there is not a great difference in the time required for the fat to reach a given degree of peroxide formation when pure oxygen and when air is used. Reduction of the oxygen concentration below that of air seems to increase this time very markedly. The values given in figure 2 indicate that the relationship between the time required to effect a certain degree of oxidation and the percentage of oxygen concentration is of a reciprocal nature, or inversely proportional. Hence,

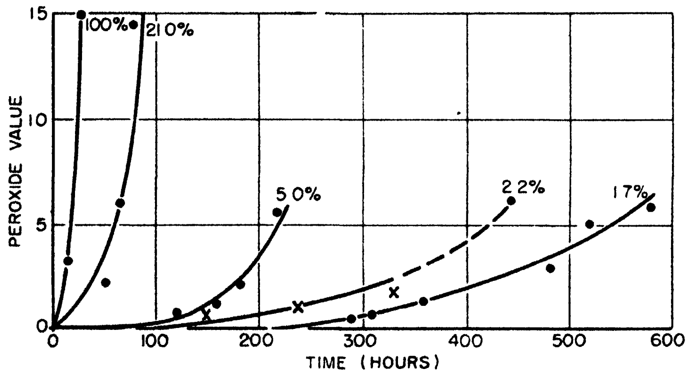

FIG. 1. The development of peroxides of butteroil at 70° in atmospheres of different oxygen concentration.

the rate of autoxidation—reciprocal of the time—is directly proportional to the oxygen concentration.

Autoxidation of the fat in dried milk. The preceding experiment was carried out under conditions wherein an excess amount of gas mixture of the given oxygen concentration was always present in each case. In the case of packed dried milks, conditions are static and the concentrations of oxygen decrease somewhat as the time of storage increases. However, for practical purposes the values of the initial oxygen concentrations are of direct interest and can be used for obtaining an approximation of the change of rate of oxidation with that of oxygen concentration.

An experiment of a type similar to that conducted with milk fat was conducted with a dried milk in which the oxygen content of each container was lowered to desired levels by single and double "gassing" procedures. The final percentage of oxygen concentration in each container was deter-

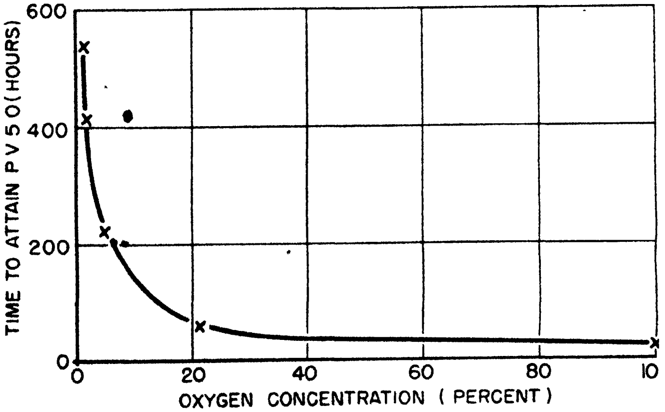

FIG. 2. The time required for the peroxides to reach a value of 5.0 in atmospheres of different oxygen concentration and at 70° C.

mined after 7 days of storage. The samples were stored at 37° C. and the time in days necessary for the production of tallowy flavors and odors was determined.

The dried milk on which these data were obtained was not of good keeping quality but the results serve to indicate the relative effect of different concentrations of oxygen in the packed containers, on the keeping quality.

It should be indicated here that the samples in the atmospheres of higher oxygen concentration were decidedly tallowy, while those in the atmospheres of low oxygen concentration were faintly tallowy, at the period of time indicated in figure 3. The samples stored in atmospheres of 1.8 per cent oxygen concentration were not judged tallowy unanimously by the judges, at the end of the period indicated.

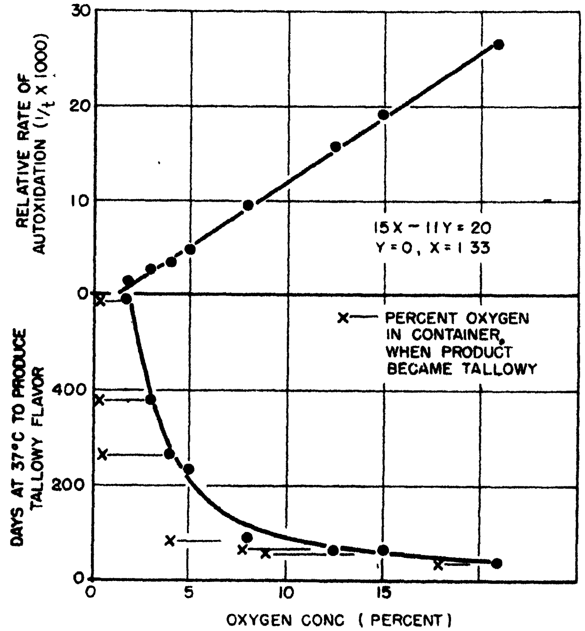

Fig. 3. The time of storage at 37° C. required to produce a tallowy flavor and the relative rate of oxidation of the fat in a dried milk at different oxygen concentration.

The data emphasize the importance of reducing the concentration of oxygen in the atmosphere of the container to less than 5 per cent in order to improve keeping quality greatly. As in the case of the experiment with butteroil, the time required for the production of a tallowy flavor is inversely proportional to the oxygen concentration. In figure 3 are shown also the reciprocals of the time periods required at different oxygen concentrations to produce a tallowy flavor. The rate of oxidation varies directly with the oxygen concentration.

However, as stated, the sample used was not of good keeping quality and more data on dried milk of all grades of keeping quality are being obtained to determine precisely the rate of spoilage in atmospheres of different oxygen concentration and to determine if these rates of autoxidation are the same for products of good and poor keeping quality.

In this connection is should be pointed out that, while theoretically the keeping quality should be infinite at zero oxygen concentration, actually it may become infinite at some low finite value of the oxygen concentration.

In the case of pure milk fat this value is approximately 0.80 per cent of oxygen by volume. To ascertain the concentration of oxygen of the atmosphere of a dried milk container which would be equivalent to this ratio, it is necessary to know the density of the dried milk and the volume of the container. The density of the dried milk was determined by evacuating thoroughly a vessel containing a known weight of dried milk and determining the amount of helium necessary to displace the air. The volume of the container was determined to the nearest milliliter. The density of the dried milk was found to be 1.29 and the volume of a 1-pound dried milk container 840 ml. The actual space occupied by the dried milk was therefore 453/1.29 or 351 ml., and the unoccupied space was 840–351 or 489 ml. The amount of fat in a pound of dried milk is approximately 118 grams, or 128 ml., and the minimal amount of oxygen necessary to produce tallowiness in this fat is 128×0.008, or 1 ml. On the basis of the unoccupied space in the container, this is equivalent to approximately 0.20 per cent.

It has been found difficult and commercially impractical to reduce the oxygen concentration of the atmosphere of dried milk containers to a concentration of 0.20 per cent. Previously published data as well as those presented here indicate that it is not necessary to attain an oxygen concentration of this value in order to increase greatly the keeping quality of the product. The present data indicate also that in the case of dried milk the amount of available oxygen can be greater than 0.20 per cent of the atmosphere of the container and yet prevent entirely the development of a tallowy flavor, presumably since the skim milk solids absorb some of the oxygen. For the dried milk on which data are given in figure 3, the value can be as high as 1.33 per cent. The value will no doubt vary with different dried milks, its magnitude depending on the rate with which the fat oxidizes and the rate and extent to which the skim milk solids absorb oxygen.

With initial concentration of oxygen greater than 1.33 per cent in the atmosphere of the container of dried milk, the rate of spoilage through oxidation varied directly with the oxygen concentration.

SUMMARY

Data have been presented which indicate that autoxidation of milk fat with 0.80 per cent by volume of oxygen is sufficient to render it inedible.

In the atmosphere of a container of dried milk this is equivalent to 0.20 per cent of oxygen. This level of oxygen concentration is difficult to attain even by "regassing" procedures. However, studies of the relative keeping quality of samples of milk fat and samples of a dried milk in atmospheres of different oxygen concentration indicate that the keeping quality increases markedly with decreases in the oxygen concentration below 5 per cent. The relationship of keeping quality in days to the initial oxygen concentration of the container atmosphere in per cent seems to be of a reciprocal nature, that is, the time required to produce tallowy flavors in packed dried milks or to reach a given peroxide value of pure milk fat, and the oxygen concentration used, are inversely proportional to one another.

The data presented seem to indicate that the rate of autoxidation of milk fat varies directly with the oxygen concentration.

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STUDIES ON KETOSIS IN DAIRY CATTLE. VIII. SPONTANEOUS RECOVERY

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In the course of investigations in this laboratory it has been observed that many cows recover from ketosis without treatment and often without any detectable change in feeding or management. If these observations are representative of other areas it is obvious that considerable caution must be exercised in attempting to evaluate the efficacy of any substance in the treatment of ketosis.

EXPERIMENTAL

The data for this report are taken from numerous records on field cases of ketosis in which the animals have recovered without treatment. Blood acetone bodies were determined by the method of Barnes and Wick (1). The method used for glucose was that of Shaffer and Somogyi (2) as modified by Somogyi (4, 5).

TABLE 1

Spontaneous recovery from ketosis (D.E. 151)

Days	Blood glucose	Blood acetone bodies (as acetone)	Comments
	mg.%	*mg.%*	
1			Parturition
10			Inappetence
16	24.4	56.34	Inappetence, decreased milk production
17	31.6	61.10	Improved appetite
19	38.7	46.31	Improved appetite
21	33.2	49.88	Inappetence
22			Improved appetite
23	48.6	20.92	Improved appetite
27	42.5	5.14	Normal appearance

Cow D.E. 151 (table 1) exhibited inappetence for approximately a week before the first blood sample was drawn, refusing all grain and eating hay sparingly. Although this animal was not treated for ketosis and environmental conditions were not altered, recovery took place within a period of 11 days as shown by the return to normal of both the blood glucose and acetone bodies. The increase in blood glucose was paralleled by an increase in food consumption.

Cow D.E. 173 (table 2) exhibited inappetence 31 days after parturition. On the basis of previous work (3) in which the blood picture was followed during the development of ketosis, it can be assumed from the marked hypo-

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TABLE 2

Spontaneous recovery from ketosis (D.E. 173)

Days	Blood glucose	Blood acetone bodies (as acetone)	Comments
	mg.%	*mg.%.*	
1	. .	.. . .	Parturition
31		. ...	Inappetence
34	15.4	69.32	Inappetence
43	32.8	35.79	Improved appetite
44	28.9	20.82	Improved appetite
46	32.3	17.54	Improved appetite
48	46.1	3.88	Normal appearance

glycemia and ketonemia at this time that the condition had been developing for some time prior to the drawing of the first blood sample. Plans had been made to treat the cow on the following day but treatment was postponed due to unavoidable circumstances. Nine days later when the second blood sample was drawn, the cow had improved so much that treatment was again postponed. Recovery took place within 18 days of the drawing of the initial sample as shown by both the blood glucose and blood acetone bodies and by the improvement in the appearance of the animal at that time.

Cow D.E. 168 developed symptoms of ketosis 17 days after parturition at which time the sodium-nitroprusside test on urine indicated ketosis. A blood sample was not taken until two days later when the cow had already shown considerable improvement. As noted in table 3, recovery was rapid, both the blood picture and the appearance of the animal returning to normal within five days. The increase in blood glucose from 34.1 to 60.8 mg. per cent within 24 hours, followed by a decrease to a normal level of 45.9 mg. per cent one day later, is of considerable interest and has been observed in a number of cases.

Cow D.E. 195 (table 4) had exhibited symptoms of ketosis for several days before the first blood sample was drawn. The appetite of this cow had shown considerable improvement on the day the initial blood sample was taken. It is apparent from the blood picture that the cow was recovering rapidly at this time. The blood glucose was almost normal but the rela-

TABLE 3

Spontaneous recovery from ketosis (D.E. 168)

Days	Blood glucose	Blood acetone bodies (as acetone)	Comments
	mg.%	*mg.%*	
1	...	..	Parturition
17	.	.	Inappetence, urine qualitative 4 +
19	34.1	28.92	Improved appetite
20	60.8	10.04	Improved appetite
21	45.9	4.30	Normal appearance

TABLE 4

Spontaneous recovery from ketosis

Cow	Days	Blood glucose	Blood acetone bodies (as acetone)	Comments
		mg.%	*mg.%*	
D.E. 195	1			Parturition
	20			Inappetence
	23			Inappetence, urine qualitative 4+
	24	43.2	35.50	Improved appetite
	25	55.6	21.30	Improved appetite
	26	51.3	3.92	Improved appetite
D.E. 257	1			Parturition
	10	25.7	17.96	Milk off flavor, cow on low plane of nutrition, grain increased
	12	42.9	6.20	

tively high blood acetone bodies indicated that the glucose had been much lower a few hours previously. As was observed in the case of D.E. 168, the hypoglycemia was over corrected for a short period followed by a decrease towards a normal level within 24 hours. The cow had not received any treatment.

Data on an interesting case of ketosis due at least in part to inanition are also given in table 4. Cow D.E. 257 had exhibited ketosis the previous year. The blood glucose level at that time was 18.98 mg. per cent and the blood acetone bodies 55.24 mg. per cent. The owner detected the same off flavor in the milk as had been observed the previous year and suspected the cow had developed ketosis. The animal did not exhibit any symptoms of ketosis although the blood glucose was 25.7 mg. per cent and the blood acetone bodies 17.96 mg. per cent. The cow had been underfed purposely in an attempt to hold down milk production but was producing 30 pounds of five per cent milk at that time. Concentrate feeding was immediately increased from three to fifteen pounds per day. As will be noted in table 4 the blood glucose and acetone bodies were almost normal within two days.

TABLE 5

Spontaneous recovery from ketosis (D.E. 77)

Days	Blood glucose	Milk production	Comments
	mg.%	*lbs.*	
1			Parturition, cow exhibited symptoms of ketosis for 34 days
34	19.5	55.3	Inappetence, lethargy
36	18.2	52.3	Inappetence, lethargy
37	20.0	51.7	Inappetence, lethargy
43	22.4	69.8	Improved appetite
47	35.0	70.7	Improved appetite
50	40.2	77.8	Improved appetite

Data are given on Cow D.E. 77 because this case is typical of many that recovered suddenly after exhibiting ketosis for some period of time. This particular animal was used for various studies including liver glycogen, insulin sensitivity and glucose tolerance tests. No treatment had been given since the thirtieth day after parturition. There was little improvement in either blood glucose or milk production from the thirty-fourth to the thirty-seventh day after parturition. A gradual improvement then took place in both. Within 13 days the blood glucose increased from 20.0 to 40.2 mg. per cent and milk production increased from 51.7 to 77.8 pounds.

DISCUSSION

Probably the majority of cows with ketosis would recover without treatment, a few quickly and many more over a longer period of time. The fact that many do recover without treatment or following treatment with substances which were later proved to be completely ineffective, emphasizes the need of caution in evaluating the results of investigations of the therapeutic value of various substances in the treatment of ketosis. To be conclusive the case should be severe and the response should be prompt. Numerous cases are highly desirable and the percentage of cases responding following treatment should be high. So far a readily available form of carbohydrate is the only substance which has been found to elicit such a response. However, ketosis could not be prevented by feeding readily available carbohydrate during the period immediately preceding parturition (3). Since ketosis began to develop within one to three days following parturition in the cases studied (3), it is difficult to subscribe to the theory that the condition is caused by a lack of soluble carbohydrate in the diet.

On the other hand, it is entirely possible that many of the milder cases of ketosis are merely cases of fasting ketosis due to an insufficient energy intake as was the case with D.E. 257 (table 4). Any deficiency which results in an impairment of appetite may be expected to produce some degree of fasting ketosis, particularly during the period immediately following parturition when the energy requirement is high. As yet, however, no deficiency other than that of a deficiency of energy in the form of carbohydrate has been proved to be responsible for the development of ketosis in cattle under farm conditions.

The reason for the spontaneous recovery which has been observed from time to time is not clear. In some cases it appeared that recovery may have been due, at least in part, to a marked decrease in milk production as recovery has been noted after milk production had decreased to a low level. In those cases where there is danger of losing the animal, cessation of milking in order to decrease the energy requirements should be helpful.

CONCLUSIONS

Data are presented on several cases of ketosis in which the cows recovered without treatment. It is emphasized that since cows do recover spontaneously, caution must be exercised in evaluating the efficacy of various substances in the treatment of ketosis in dairy cattle.

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RELATION OF CORN AND ALFALFA SILAGE TO THE QUALITY OF CHEESE AND ITS CAROTENE AND VITAMIN A CONTENT*

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INTRODUCTION

There seems to be a general belief among cheese makers that the use of grass (alfalfa) silage for producing cheese milk is undesirable. This is particularly true with respect to Swiss cheese where certain bacteria that may be associated with silage are blamed for low quality in the product (1, 4, 5, 6, 7, 9). An opportunity to investigate the quality of cheese as related to type of silage arose during experiments on the effect of feeding corn and alfalfa silages upon the vitamins of winter milk (8). Several lots of cheddar cheese were made from these milks during January and February of 1944, and after suitable periods of curing were sampled and graded as to quality and analyzed for carotene and vitamin A. Before the analyses could be made a satisfactory procedure for the chemical determination of carotene and vitamin A had to be developed.

EXPERIMENTAL SILAGES AND MILKS

The alfalfa silage was made with 200 pounds of corn and cob meal per ton added to the forage as it was run through the silage cutter. The alfalfa was of good quality and contained about 22 per cent dry matter at the time of ensiling. When fed during the months of November to February inclusive, the silage averaged 245 µg. carotene per gram dry matter.

Hybrid corn was used for the making of the corn silage. It was quite green and succulent and averaged about 22.5 per cent dry matter. The silage when fed averaged 72 µg. of carotene per gram of dry matter.

The milks used for the experimental cheese were obtained from three lots of cows of five animals each. The animals were selected so that the period of lactation and butterfat production would be approximately equal for each lot. All animals were fed grain and hay and in addition Lot I was given corn silage as the silage part of the ration, Lot II received both corn silage and alfalfa silage, and Lot III was given only alfalfa silage.

The amount of carotene ingested by the animals in the three lots varied according to their rations. Lot I received approximately 400 mg. per cow

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TABLE 1

Feed and milk data

Items	Lot I	Lot II	Lot III
	Ration		
	lbs./day	*lbs./day*	*lbs./day*
Grain	8.5	8.7	8.7
Hay	14.0	14.0	14.0
Alfalfa silage		20.2	40.8
Corn silage	40.3	20.2	
	Carotene ingested		
	mg./day	*mg./day*	*mg./day*
From silage	289	659	1039
From hay	125	125	125
	Milk production, 4% fat basis		
	lbs.	*lbs.*	*lbs.*
Total for period	10,780	10,512	10,250
Ave./cow/day	25.67	25.02	24.41
	Carotene and vitamin A content of milk		
	μg./gm. of butterfat	*μg./gm. of butterfat*	*μg./gm. of butterfat*
Carotene, ave.	4.58	5.39	5.74
Vitamin A, ave.	5.56	5.89	6.94

per day, Lot II received about 800 mg. per cow per day and Lot III received about 1200 mg. per cow per day. As would be expected, high carotene ingestion was reflected in the carotene and vitamin A content of the milks, but the correlation was not directly proportional.

Milk production on a 4 per cent fat-corrected basis showed no practical difference among the three lots of cows.

The data on the composition of the ration, the amount of carotene ingested by each cow per day, the milk production on a fat-corrected basis, and the carotene and vitamin A content of the milk-fat produced are presented for each lot in table 1.

EXPERIMENTAL CHEDDAR CHEESE

Preparation and grade. Experimental cheese was made from each of the three lots of raw milk on eight occasions during January and February of

TABLE 2

Average scores of cheese at three months

Lot	Silage	Flavor	Body and texture	Color and finish	Total
I	Corn silage	37.5	28.0	24.9	90.4
II	Corn and alfalfa	37.9	28.4	24.9	91.2
III	Alfalfa silage	38.0	28.3	25.0	91.3

1944. After a three months' curing, the cheeses were scored by members of the Dairy Industry Department. The results showed practically no difference in quality among the three lots of cheese. There did seem to be a slight margin in favor of the alfalfa silage lot. The scores based on flavor, body, texture, color, and finish were as follows: 90.4, 91.2 and 91.3, for Lots I, II, and III, respectively. Complete data are given in table 2.

METHOD FOR THE DETERMINATION OF CAROTENE AND VITAMIN A IN CHEESE

The method used for butter by Berl and Peterson (2) was modified to make it suitable for the analysis of cheese. Calibration curves were constructed for both carotene and vitamin A by means of standards as described in their paper. It has been found desirable in our experience for each analyst to standardize his own curve in order to avoid personal variations. Only a brief statement covering the essential points in the procedure will be given. For details see the original paper.

A 10-gm. sample is minced and saponified in a methanol-potassium hydroxide solution. After cooling and diluting with water, the nonsaponifiable matter is extracted with several portions of ether. The extract is washed with water and dried over sodium sulfate. The ether is removed over a water bath under reduced pressure, replaced with Skelly-Solve B and the extract is made up to 50 ml. in a volumetric flask. A 25-ml. aliquot is extracted three times with 10-ml. portions of 94 per cent diacetone to remove noncarotene pigments, the residue is washed with water, made up to 25 ml. in a volumetric flask and read for carotene in the Evelyn colorimeter, at

TABLE 3

Check on analytical method by means of added carotene and vitamin A

Type of cheese	Carotene or vitamin A per 10 gm. sample	Carotene or vitamin A added	Carotene or vitamin A found	Recovery
	Carotene			
	µg.	µg.	µg.	%
Brick	12.2	9.4	20.7	96
Cheddar	14.3	9.4	22.9	96
Cheddar	17.0	28.2	42.0	93
Swiss	16.8	19.3	33.5	93
Swiss	18.8	19.0	36.0	95
	Vitamin A			
	µg.	µg.	µg.	%
Brick	19.5	12.6	30.6	95
Cheddar	27.4	12.6	38.1	95
Cheddar	30.2	28.0	56.2	96
Cheddar	19.8	5.2	25.2	101
Swiss	31.5	35.1	62.5	94

440 mμ. For the determination of vitamin A a 5-ml. aliquot of the original extract is placed in a colorimeter tube, evaporated under reduced pressure and the residue redissolved in 1 ml. of chloroform. This is treated as usual with the antimony trichloride reagent and the resultant blue color is read at 620 mμ.

The method was found to give approximately 95 per cent recoveries of amounts of both carotene and vitamin A added to the cheese prior to saponification. Data are given in table 3.

TABLE 4

Distribution of carotene and vitamin A in cheese-making

Date	Lot	Milk			Cheese			Whey			Recovery in products	
		Lbs.	Carotene	Vitamin A	Lbs.	Carotene	Vitamin A	Lbs.	Carotene	Vitamin A	Carotene	Vitamin A
			*μg/lb.**			*μg./lb.*			*μg./lb.*		%	%
1/ 6/44	I	63	78	96	7.0	635	784	56.9	8.1	9.1	100	99
	II	63	94	98	6.7	780	815	55.9	9.3	8.6	97	98
	III	63	76	111	6.2	717	1020	56.4	8.9	7.7	103	96
1/12/44	I	54	84	112	5.9	690	920	48.1	6.1	9.5	96	97
	II	54	92	122	5.7	648	960	47.6	7.3	9.1	81	90
	III	54	81	122	5.4	658	938	48.5	6.3	7.2	87	82
1/18/44	I	52	82	99	5.7	630	788	45.4	6.0	6.8	91	93
	II	52	95	119	5.6	753	734	45.8	8.8	6.8	93	72
	III	52	97	99	5.2	708	1020	45.7	9.2	9.9	81	110
1/26/44	I	52	77	104	5.2	644	914	45.9	8.2	9.1	98	95
	II	52	87	109	5.4	671	806	45.9	6.8	8.1	87	84
	III	52	76	100	5.2	558	752	46.3	6.0	7.2	80	81
2/ 2/44	I	52	67	104	5.5	626	882	45.7	4.5	7.7	104	96
	II	52	91	120	5.5	694	960	45.7	5.6	8.1	86	90
	III	52	90	113	5.2	716	1180	46.6	6.4	7.2	86	110
2/ 3/44	I	52	(67)	(104)	5.4	544	828	46.7	5.2	7.7	91	96
	II	52	(91)	(120)	5.5	694	906	45.7	6.4	8.6	86	86
	III	52	(90)	(113)	5.2	704	978	46.6	6.0	6.8	84	92
2/ 9/44	I	52	69	97	5.5	640	828	46.4	6.4	11.3	106	100
	II	52	83	110	5.4	716	928	46.8	8.9	11.7	99	97
	III	52	85	123	4.9	685	1040	44.8	7.6	9.5	84	86
2/10/44	I	52	(69)	(97)	5.3	566	834	46.0	5.3	9.0	90	96
	II	52	(83)	(110)	5.2	680	840	45.6	8.1	10.4	90	95
	III	52	(85)	(123)	5.0	694	1060	45.2	8.4	8.2	86	89

* Figures in parentheses are those for the day immediately preceding as analyses were run on only the first of the two consecutive days.

DISTRIBUTION OF CAROTENE AND VITAMIN A IN CHEESE MAKING

The carotene and vitamin A contents were determined on the milk, cheese and the whey. The procedure for milk and whey was by the method of Dornbush, Peterson and Olson (3). The distribution of vitamin A potency of milk in cheese making was shown to be as follows: 85 per cent was retained in the cheese, 7 per cent accompanied the whey and 8 per cent was unaccounted for. These figures apply to both carotene and vitamin A.

Losses probably occurred in the adherence of milk fat on containers and equipment during processing. The three lots of milk gave cheese of increased vitamin A potency in the order Lot I, Lot II and Lot III. It was evident that alfalfa silage was superior to the corn silage or the corn silage–alfalfa silage mixture. Data are given in table 4.

Effect of storage. After the original analysis made immediately upon production, repeated analyses were made of the cheeses at intervals of 8 weeks, 12 weeks and 42 weeks. The carotene content of the samples ranged from 1.20–1.57 µg./gm. for Lot I, 1.43–1.79 µg./gm. for Lot II, and 1.45–1.79 µg./gm. for Lot III. The vitamin A content ranged from 1.68–2.05 µg./gm., 1.62–2.20 µg./gm., and 1.66–2.53 µg./gm. for Lots I, II, and III, respectively. Data are given in table 5.

TABLE 5

Changes in carotene and vitamin A content of cheese in storage

Lot No.	Carotene content after				Vitamin A content after			
	0 weeks	8 weeks	12 weeks	42 weeks	0 weeks	8 weeks	12 weeks	42 weeks
		µg./gm.				*µg./gm.*		
I	1.37	1.36	1.40	1.44	1.87	1.86	1.86	1.85
II	1.55	1.66	1.67	1.72	1.98	1.98	1.98	1.97
III	1.50	1.62	1.63	1.67	2.21	2.24	2.28	2.26

Each value represents the average of 8 samples.

No significant change in vitamin A potency occurred during nearly a year in storage. The carotene content showed a slight increase while the vitamin A content was quite constant. As possible explanations for the apparent increase in carotene content several factors such as drying of the cheese, a change in the characteristics of certain of the noncarotene pigments, etc., may be considered, but the effect was too small to warrant extended investigation.

SUMMARY

Eight lots of cheese made from three types of silage milk were studied with respect to quality and vitamin A potency.

Cheese made from milk produced on an alfalfa silage ration equalled or excelled in quality and vitamin A potency, cheese made from milk produced on a corn silage ration.

A method for the determination of carotene and vitamin A in the cheese was devised.

The distribution of vitamin A potency in cheese-making was shown to be as follows: 85 per cent was retained in the cheese, 7 per cent was found in the whey and 8 per cent remained unaccounted for. A pound of milk, which contained on the average 83 µg. of carotene and 109 µg. of vitamin A, gave cheese containing 69 µg. of carotene and 93 µg. of vitamin A. The whey accounted for 6.5 μg. of carotene and 7.5 μg. of vitamin A; thus leaving about 7.5 μg. of carotene and 8.5 μg. of vitamin A unaccounted for.

The vitamin A potency of cheese during curing and storage was practically unchanged over almost a year.

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Credit is due J. J. Stefaniak for making the carotene and vitamin A analyses of the milks reported in table 4.

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THE VITAMIN CONTENT OF MARE'S MILK*

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According to Associates of Rogers (1), mare's milk is commonly used as a food in some sections of Western Asia. It is not used for human food in this country, but one may very properly consider the feasibility of using mare's milk for infant feeding, since its composition resembles that of human milk more nearly than cow's or goat's milk, which is commonly used for infant feeding. This is particularly true with respect to milk sugar. According to Gardner and Fox (6), and Brown and co-workers (3), human milk contains 7.38 per cent and 6.79 per cent of lactose, respectively, while Linton (18) and Hildebrandt (8), respectively, report mare's milk contains 6.14 per cent and 6.42 per cent. Furthermore, Turner (29) found that mare's milk is digested (in vitro) more readily than either cow's or goat's milk. However, at the moment far more interest is centered upon the nutritive value of mare's milk for raising foals. The observation at Massachusetts Agricultural Experiment Station that the body weight of Percheron foals when they are weaned varied from 685 to 830 pounds, naturally raised a question concerning whether the variability in weight results primarily from differences in the milk-producing capacity of the mares or in the quality of the milk. A review of the available literature produced relatively little information concerning the vitamin content of mare's milk. Furthermore, one should not attempt to estimate the vitamin content of mare's milk from data concerning cow's milk even when, as at Massachusetts Agricultural Experiment Station, the mares and cows feed in the same pasture and on hay grown in the same or similar fields, for cows are ruminants and mares are nonruminants. Wegner, Booth, Elvehjem, and Hart (31), Hunt, Kick, Burroughs, Bethke, Schalk, and Gerlaugh (15) and others have shown that cattle can synthesize riboflavin in the rumen. Furthermore, Bechdel, Honeywell, Dutcher, and Knutsen (2) found that the microflora of the rumen enabled cows fed a vitamin B deficient ration to produce milk of normal vitamin B content. Also McElroy and Goss (19) found appreciable amounts of thiamine in the milk of a fistulated cow whose rumen contained no thiamine; but mares do not have a rumen in which to synthesize vitamins. In view of the paucity of data, this study was conducted to accumulate additional information regarding the vitamin content of mare's milk.

DESCRIPTION OF MARES

At the time the study was undertaken, only three Percheron brood mares,

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Bay State Laurette, Bay State Laura and Bay State Lady Lou were available as a source of milk.

Data concerning the age, weight, and breeding records of the mares in question are reported in table 1.

It will be noted that the body weight of Laura and Lady Lou are somewhat greater than that of Laurette, but this was to be anticipated since the latter worked regularly, often in the hot sunshine, sweating profusely while the other two were idle in a cool barn. The stage of lactation reported in table 1 pertains to the beginning of the experiment. When comparing the results of this study, it should be remembered that Laura is the mother of Laurette, and that Lady Lou and Laurette are half-sisters of the same age. During the period of this experiment, the mares and foals were maintained on a good pasture during the nights and were fed grain and hay in the barn during the day. Each mare received 3–4 quarts of crushed oats and 5 or 6 large ears of thoroughly matured, dry corn daily.

TABLE 1

Data concerning some of the physical and physiological aspects of the mares studied

Name	Breed	Age	Weight	No. of foal	Stage of lactation	Bred	Activity
		yrs.	*lbs.*		*mo.*		
Bay State Laurette	Percheron	7	1640	First	3	No	Works constantly
Bay State Laura	"	14	1800	Tenth	2	Yes*	Worked before foaling, idle since
Bay State Lady Lou	"	7	1900	Fourth	4	Yes*	Idle entire life
Dutchess	Saddle pony	9	1040	?	1.5	No	Ridden every day

* No recurrence of oestrus.

COLLECTION OF MILK

A pint or more of milk was required for the various assays. Hence it was necessary to separate the foals from the mares for an hour or more previous to collecting the sample. Since some of the assays were to be made by microbiological procedures, it seemed preferable to keep the bacterial content of the milk as low as possible. Accordingly, the milk was drawn directly into one-half-pint, sterilized milk bottles which were immediately capped.

It was of course recognized that the composition of the milk would not be truly typical for the animal in question unless the entire volume of milk was removed from the udder and thoroughly mixed before sampling, but since the foals were nursing, that procedure could not be followed, and only enough milk was drawn for assay purposes. Thus all samples of milk from the Percheron mares represented the fore-milk. It was drawn just prior to or, in case the mare refused to "give down her milk," simultaneously with the foals nursing.

The freshly drawn mare's milk was very white with a slightly bluish tinge and thus its appearance was more like human milk or cow's skimmed milk than normal cow's or goat's milk. When the milk was drawn from the mare's udder or was subsequently poured from bottle to bottle, it foamed excessively due probably to a higher albumin content than is characteristic of cow's milk. The mare's milk had a sweetish taste, which perhaps might have been anticipated from its high lactose content.

During the progress of the study, a privately owned saddle mare, Dutchess, lost her 6-week-old foal by tetanus (lockjaw). It was necessary to milk the mare while terminating lactation. Assays were made of her milk, which was always cream-colored, but the results should be considered as applying to abnormal milk, since the intervals between drawing the milk increased and the volume of milk decreased. Samples 2 and 3 were obtained from the same milking. Sample 2 is representative of one pint of fore-milk and sample 3 represents one and one-half quarts of the last milk drawn. Likewise sample 4 represents the fore-milk and sample 5 the last portion of the milk drawn 72 hours after samples 2 and 3. Sample 6 was drawn the following day (24 hours later) and was representative of all the milk obtained. Sample 7, which was drawn more than two days (52 hours) later, was deep cream color and was representative of the one pint of milk, which was the total quantity that could be obtained.

EXPERIMENTAL

The milk was taken directly to the laboratory and assayed for ascorbic, nicotinic and pantothenic acids, riboflavin, thiamine, bacteria, fat, size of fat globules, and total solids. The bacterial and ascorbic acid assays were started within approximately 15 minutes after the milk was drawn from the mares. The remaining assays were started within the next hour. The reduced ascorbic acid was assayed by Sharp's (26) rapid method. The microbiological method of Krehl, Strong, and Elvehjem (16) was employed for determining the amount of nicotinic acid. The Neal and Strong (21) microbiological method was used for pantothenic acid. Riboflavin was assayed by the Holmes and Jones (12) method and thiamine by a modification of the Hennessy and Cerecedo (7) procedure. The number of bacteria was estimated in accordance with "Standard Methods for the Examination of Dairy Products" (28), and fat was determined by the Babcock (17) method, modified to the extent that only 15 cc. of acid were used. The size of the fat globules was measured by an adaptation of the hanging drop procedure, and the Mojonnier and Troy (20) method was followed for determining the total solids.

RESULTS

Forty samples of milk from 3 Percheron mares and a saddle pony, were assayed for ascorbic acid, riboflavin, bacteria, fat, size of fat globules, and total solids. Nearly all the samples were assayed for nicotinic acid and thiamine, but only three samples were assayed for pantothenic acid. The results of these assays are reported in detail in table 2. For convenience, these results are discussed under the appropriate headings.

Ascorbic acid. The ascorbic acid content of the individual samples of milk from each mare varied. The average values were 13.7 for Laurette, 12.2 for Laura, and 12.7 mg. per liter for Lady Lou. Judged by these values, the milk of Percheron brood mares contained only about two-thirds as much reduced ascorbic acid as that previously reported (11) for cows which consumed forage produced on the same land and under essentially the same conditions as the hay and grass consumed by the mares. However, Sigrist (27) claims that the vitamin C content of mare's milk is not dependent upon the age or the type of ration but varies with individual animals.

Nicotinic acid. The nicotinic acid content of normal milk varied from 0.53 mg. for sample 7 from Laurette to 0.83 mg. per liter for sample 8 from Laura. The average values were 0.67 mg. for Laurette, 0.77 mg. for Laura and 0.72 mg. per liter for Lady Lou. The amount of nicotinic acid in the milk from Dutchess varied from 0.53 mg. to 0.94 mg. with an average of 0.72 mg. per liter, but, as mentioned before, this milk was obtained during the cessation of lactation. These values are somewhat lower than 1.1 mg. per liter obtained for milk (9) from the college herd of cows during the late summer.

Pantothenic acid. Due to unforeseen developments, it was possible to assay only three samples of mare's milk for pantothenic, namely sample 1 from each of the three Percheron mares. The values obtained were 3.47 mg. for Laurette, 2.55 mg. for Laura and 2.28 mg. per liter of milk from Lady Lou. These data are too meager to permit any general conclusions concerning the pantothenic acid content of mare's milk, but it is of interest to note that the average value, i.e., 2.77 mg. per liter, is somewhat lower than 3.66 mg. per liter previously reported (10) for the milk from the college herd of cows.

Riboflavin. The riboflavin content of the milk from the Percheron brood mares was quite variable. The average values obtained were 0.11 mg. for Laurette, 0.13 mg. for Laura, and 0.09 mg. per liter for Lady Lou. These values are less than one-tenth that obtained for cow's milk previously studied (13) in this laboratory. However, they are in agreement with the findings of Rasmussen, Bogart, and Maynard (24), who state that limited studies by the fluorometric method indicated that the lactoflavin content of mare's milk is very low. Later, Rasmussen (25) reported that the riboflavin content of mare's milk was about one-tenth the amount found in cow's milk.

TABLE 2

Results of assays of mare's milk

Sample No.	Ascorbic acid	Nicotinic acid	Riboflavin	Thiamine	Bacteria	Fat	Size of fat globules	Total solids
Bay State Laurette								
	mg./l.	*mg./l.*	*mg./l.*	*mg./l.*	*per cc.*	%	μ	%
1	14.5		0.08		150	0.6	3.0	9.73
2	6.5		0.07		420	0.7	3.0	9.87
3	13.5	0.75	0.09	0.45	1340	1.3	3.0	10.34
4	10.0	0.64	0.08	0.52	500	0.8	3.0	10.12
5	17.5	0.56	0.11	0.49	990	0.4	3.0	9.84
6	16.5	0.58	0.11		880	1.8	4.0	10.80
7	14.5	0.53	0.14		550	1.3	3.0	9.86
8	16.5	0.62	0.12	0.44	150	1.0	4.0	9.76
9	14.5	0.81	0.13	0.42	260	1.1	2.5	10.17
10	14.0	0.73	0.17	0.46	810	1.1	3.0	10.02
11	12.5	0.81	0.15	0.41	60	0.8	3.0	10.11
Average	13.7	0.67	0.11	0.46	555	1.0	3.1	10.06
Bay State Laura								
1	11.5		0.05		60	0.8	4.0	9.67
2	12.0		0.11		950	1.3	4.0	10.41
3	10.5	0.80		0.32	630	0.7	4.0	9.63
4	11.0	0.81	0.07	0.46	130	1.5	4.0	10.40
5	15.0	0.81	0.15	0.46	170	0.8	4.0	10.08
6	17.0	0.75	0.09		160	1.3	3.5	10.41
7	13.5	0.66	0.16		410	1.5	3.0	10.25
8	10.5	0.83	0.20	0.42	190	1.8	3.0	10.57
9	10.0	0.77	0.15	0.38	340	0.8	3.0	9.88
10	12.0	0.80	0.15	0.42	140	1.1	3.0	10.13
11	11.5	0.72	0.19	0.38	130	1.0	3.0	10.22
Average	12.2	0.77	0.13	0.41	301	1.1	3.5	10.15
Bay State Lady Lou								
1	15.5		0.06		80	1.6	2.5	10.41
2	9.0		0.07		610	1.8	3.5	10.88
3	10.0	0.75	0.07	0.40	230	1.9	4.0	10.87
4	10.5	0.68	0.07	0.33	510	1.1	3.5	9.96
5	15.5	0.81	0.09	0.34	380	1.2	4.0	10.28
6	20.0	0.65	0.09		310	2.5	4.0	11.21
7	13.0	0.68	0.12		210	1.5	3.5	10.25
8	15.5	0.67	0.08	0.25	20	2.5	4.0	10.63
9	10.0	0.71	0.14	0.30	270	1.0	3.0	9.74
10	12.5	0.77	0.13	0.30	50	1.8	4.0	10.34
11	8.5	0.76	0.09	0.28	170	0.9	3.5	9.84
Average	12.7	0.72	0.09	0.31	258	1.6	3.6	10.40
Dutchess								
1	15.0*	0.65	0.15			6.5	5.0	13.74
2	16.5	0.53	0.22		30	0.4	2.0	8.42
3	10.5	0.53	0.28			1.6	7.0	9.20
4	22.0	0.80	0.36	0.24	900	2.5	3.0	9.29
5	23.5	0.93	0.36	0.25	200	9.5	9.0	17.49
6	16.0	0.69	0.26	0.24	900	1.5	4.0	10.47
7	17.0	0.94	0.22		190	7.1	5.0	16.33
Average	17.2	0.72	0.26	0.24	444	4.2	5.0	12.13

* For details regarding samples drawn during the rapid cessation of lactation see page 165.

Thiamine. The average values for seven samples of milk from each of the Percheron mares were: 0.46 mg. for Laurette, 0.41 mg. for Laura, and 0.31 mg. per liter for Lady Lou. Judged by these values for individual animals, mare's milk contained slightly more thiamine than 0.33 mg. per liter which was found in raw, mixed, herd milk (9) produced by Ayrshire, Holstein, Guernsey, and Jersey cows, and slightly less than 0.45 mg. per liter previously reported as an average value for goat's milk (14).

Bacteria. The average number of bacteria in the mare's milk was 550 for Laurette, 300 for Laura and 260 per cc. for Lady Lou. These relatively small numbers of bacteria in freshly drawn mare's milk indicate that it, like cow's milk, is probably sterile or nearly so when drawn by the foal directly from the udder. If this assumption is tenable, one must naturally conclude that if foals synthesize vitamins in the intestinal tract, such synthesis is not initiated by the bacteria present in the mare's milk supplied to the foal.

Fat. The fat content of all the samples of milk from the Percheron mares was extremely low as compared with values reported by Overman, Sanmann, and Wright (22) which ranged from 3.55 per cent for Holstein to 5.19 per cent for Guernsey milk. The average values were 1.0 for Laurette, 1.1 for Laura and 1.6 per cent for Lady Lou. Possibly the low fat content may have been a cause for the very white appearance of the milk. However, it should be noted that the mare's milk used in this study was the fore-milk and Van Slyke (30) and others have shown that during milking the percentage of fat increases and unless the udder is carefully stripped and the total milk thoroughly mixed, the sample will not be truly representative with respect to the fat. In fact, in one instance the fore-milk contained less than one-tenth as much fat as the last portion (stripping). Linton (18) found that the fat content of mare's milk increased with the length of time the foal was separated from the mare. In fact, he claims that if the foal is kept from the mare for several hours, the fat content of the milk will increase to a level higher than is well tolerated by foals and he points out that the empirical procedure of milking out a mare that has been working or otherwise separated for several hours from her foal, has a sound scientific basis.

Size of fat globules. The size of fat globules (table 2) represents the average obtained for each sample. While the variation in size is not as large as that reported for cow's milk fat globules (1), i.e., 0.10 μ to 22.0 μ, the average values 3.1 μ for Laurette, 3.5 μ for Laura and 3.6 μ for Lady Lou are essentially the same as the average size of fat globules in cow's milk; namely, 3.0 μ. They are larger than 2.0 μ reported (1) for goat's fat globules.

Total solids. The total solids in the milk varied somewhat for each animal. The average values were 10.06 for Laurette, 10.15 for Laura and 10.40

per cent for Lady Lou. These values are defintely lower than the value of 12.57 per cent reported by Eckles and Shaw (5) for Shorthorn cow's milk and 12.00 per cent, 12.72 per cent, 14.49 per cent, and 14.70 per cent for milk (22) from Holstein, Ayrshire, Guernsey, and Jersey cows respectively.

Hydrogen ion concentration. The pH values obtained for the forty samples of mare's milk varied for the different animals and also varied from day to day for the same animal. The range was from pH 6.66 to 6.98 with an average of 6.81 for normal milk from the Percheron mares. The milk obtained from the saddle mare during the cessation of lactation had a higher pH reading. The value 6.81 is definitely higher than pH 6.37 previously reported (14) for goat's milk, slightly higher than pH 6.6 computed (1) for cow's milk, but less than 6.97 found by Davidsohn (4) for human milk.

Mare's milk during cessation of lactation. The seven samples of milk obtained from Dutchess during the cessation of lactation differed from the milk produced by the Percheron mares. The average values were ascorbic acid, 17.2; nicotinic acid, 0.72; riboflavin, 0.26; and thiamine, 0.24 mg. per liter, fat, 4.2 per cent; size of fat globules, 5.0 μ and total solids, 12.13 per cent. Of these values, the ascorbic acid, riboflavin, fat, size of fat globules, and total solids were greater than for the milk from the Percheron mares; the nicotinic acid was about the same; but the thiamine content was less than that of the other mares. To what extent these differences were brought about by differences in size, breed, and stage of lactation is not known. The very rapid cessation of lactation may have been a major cause of some of the differences in the composition of the milk, for Palmer and Eckles (23) and others have found that as cows and women approach the end of lactation, the protein and fat content of their milk increases.

SUMMARY

Forty samples of milk were obtained from three Percheron mares during the normal lactation and from a saddle pony during very rapid cessation of lactation. The milk was very white with a bluish tinge, foamed excessively and had a sweetish taste. The average values for mare's milk during normal lactation were as follows: ascorbic acid, 12.9 mg.; nicotinic acid, 0.72 mg.; pantothenic acid (three samples), 2.77 mg.; riboflavin, 0.11 mg.; thiamine, 0.39 mg. per liter; fat, 1.2 per cent; fat globule size, 3.4 μ; total solids, 10.20 per cent. Of these values ascorbic and nicotinic acid, riboflavin, fat, and total solids were lower than the corresponding values for cow's milk, but the thiamine values were higher.

The milk obtained from the saddle pony during the period of rapid cessation of lactation contained more ascorbic acid, riboflavin, fat, and total solids, and had larger fat globules than was found for the other three mares experiencing normal lactation; the amount of thiamine was much less.

The greatest difference in vitamin content between mare's and cow's milk was in riboflavin; the former having only about one-tenth of the latter.

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PROPIONIC ACID, SODIUM PROPIONATE AND CALCIUM PROPIONATE AS INHIBITORS OF MOLD GROWTH.
II. STUDIES PERTAINING TO THE ACTIVE AGENT RESPONSIBLE FOR THE INHIBITORY EFFECT OF THE PROPIONATES*

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Recent studies (4, 9, 11) have shown that aqueous solutions of sodium or calcium propionate, when used in treating parchment paper for wrapping prints of butter, were effective in restraining the growth of mold on the butter surface.

Investigators in several branches of biology have been interested in the action of organic acids and their salts on microorganisms and many have sought to determine the active agent responsible for the physiological responses observed. A more detailed review of the literature on this subject has been reported (10). Variations in the concentration of ions and in undissociated molecules of different acids evoking similar responses in organisms studied have been noted (1, 14, 16). Marked differences in inhibitory qualities of different acids at the same pH for several species of molds, bacteria and yeasts have been observed (3, 7, 8, 15). Attempts to depress ionization of organic acids have resulted in increased inhibition of the organisms studied (2, 5, 6, 12, 13). Wynne (17) used thirty representative inorganic and organic acids in a study of their relation to the growth of *Cl. acetobutylicum.* His results indicated that certain lower members of the fatty acid series owed their toxicity primarily to certain critical concentrations of hydrogen ions. However, in the case of the higher members of the series other factors influenced the inhibiting pH.

In short, the growth inhibitory action of organic acids for various organisms has been attributed to each component of the dissociated acid system. From the equation for acid dissociation,

$$\text{Ka} = \frac{(\text{H}+)\ (\text{A}-)}{(\text{HA})},$$

it is apparent that the concentration of unionized acid may be increased by two methods: 1) by increasing the hydrogen ion concentration; 2) by in-

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creasing the anion. In an aqueous solution of sodium propionate or calcium propionate it may be assumed that hydrogen ions, propionate ions, sodium or calcium ions, and undissociated acid exist. Further addition of hydrogen ions or propionate ions to such a system will drive the equilibrium in the direction of increased unionized acid. By rearrangement, the above equation may be written,

$$(\mathrm{HA}) = \frac{(\mathrm{S})\ (\mathrm{H}+)}{\mathrm{Ka} + (\mathrm{H}+)}$$

Thus, if the pH and the concentration of total acid (S), *i.e.*, the concentrations of total material containing the acid's main group in the form of ions or ionogens, are known, the concentration of the undissociated acid may be calculated. If any one of the components of the system is solely responsible for inhibition of growth of a particular organism, growth should be inhibited at a specific concentration of that component.

In this report the effect of the various components of the propionic acid dissociation system on the growth of certain molds is described.

EFFECT OF PROPIONIC ACID IN A NUTRIENT MEDIUM ON THE GROWTH OF SEVERAL SPECIES OF MOLDS

Preliminary studies indicated that different molds varied in their resistance to the inhibitory action of the propionates. This was investigated further using several different concentrations of propionic acid in potato dextrose agar medium (4.5 gm. Bacto dehydrated potato-dextrose medium per 100 ml. of solution). This basal medium was used to prepare a series of media in which propionic acid concentrations ranged from 0.006 M to 0.02 M. Prior to the addition of the acid, the medium was adjusted to pH 7.0. After the acid was added, a sample of each medium of the series was back titrated electrometrically to pH 7.0 to check the concentration of propionic acid. Spores from the respective molds were suspended in distilled water. Then 0.05-ml. quantities of these suspensions were placed in Petri dishes. The dishes then were poured in duplicate with each medium. Incubation was at room temperature. Several species of mold were used including *Penicillium intracatum, Alternaria tenuis, Hormodendrum cladosporioides, Stemphylium congestum* and a *Cladosporium species.* Figures 1 and 2 show the appearance of representative plates which illustrate the effect of propionic acid on growth of these molds and the variation in resistance to its inhibitory effect by different molds.

EFFECT OF SODIUM PROPIONATE IN A MEDIUM AT VARIOUS pH LEVELS ON THE GROWTH OF MOLD

Potato-dextrose-agar medium containing two per cent sodium propionate was adjusted to various pH levels, lactic acid being used to make the adjustments. A number of preliminary trials were made using several molds to

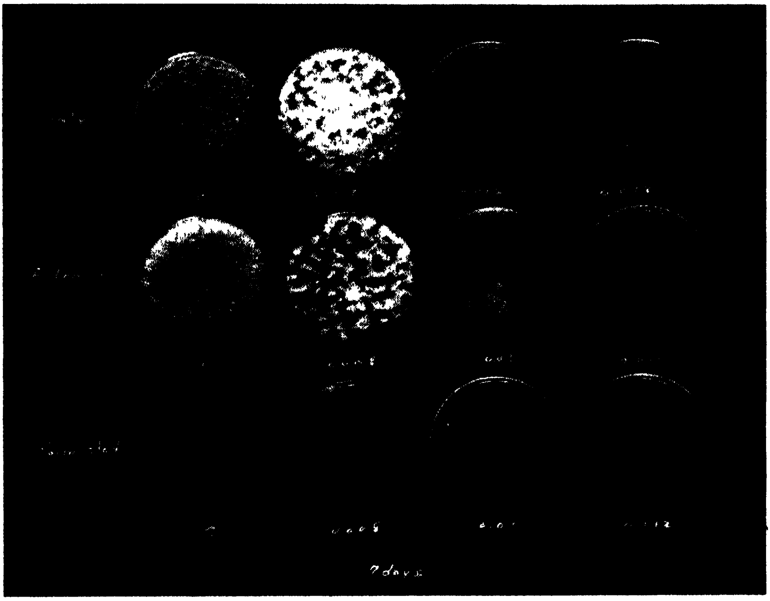

FIG. 1. This photograph shows the appearance of representative plates from Experiment I. Four plates each of *Penicillium intracatum, Alternaria tenuis,* and *Hormodendrum cladosporioides* are shown. The plates marked "C" contained no propionic acid.

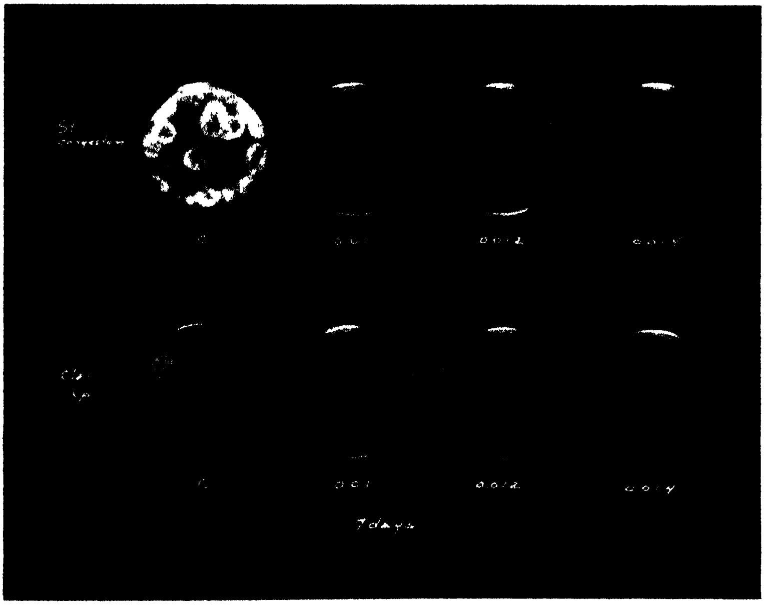

FIG. 2. This photograph shows the appearance of representative plates from Experiment I. Four plates each of *Stemphylium congestum* and *Cladosporium species* are shown. The plates marked "C" contained no propionic acid.

TABLE 1

The effect of reducing the pH of a medium containing two per cent sodium propionate on the growth of mold

Concentration of sodium propionate in potato-dextrose medium	pH of medium	Relative amount of growth of *H. cladosporioides* after 7 days
per cent		
0.0	5.25*	7 +
2.0	6.96*	6 +
2.0	6.73	5 +
2.0	6.63	4 +
2.0	6.49	3 +
2.0	6.38	2 +
2.0	6.27	1 +
2.0	6.18	+ (–)
2.0	6.11	–
2.0	6.09	–
2.0	5.85	–

Note: Lactic acid used to reduce pH.
* No lactic acid added.

determine the pH range within which a gradual restraint of growth could be demonstrated. In the initial trial, abundant growth was obtained in the control medium (no propionate added) and in the medium containing two per cent sodium propionate without any addition of lactic acid. The pH of

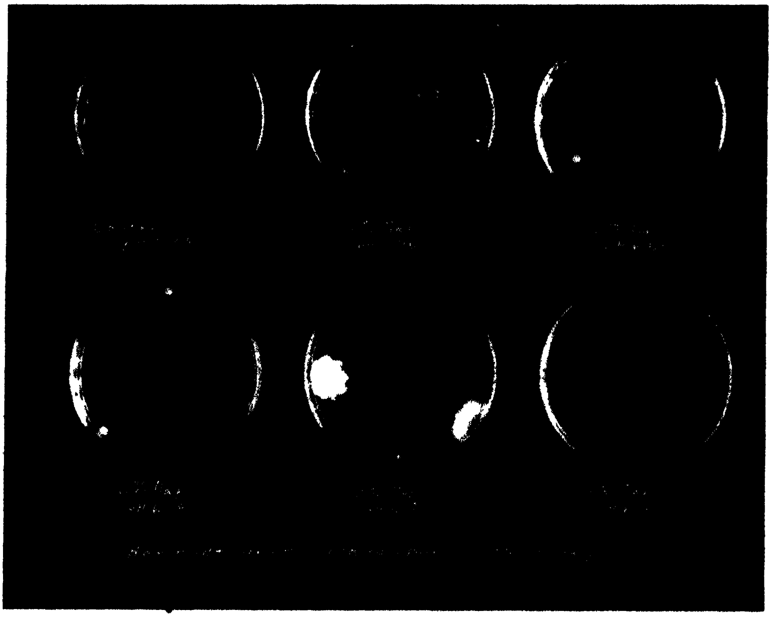

Fig. 3. This photograph shows the appearance of representative plates from Experiment II. The growth of *Hormodendrum cladosporioides* on six plates containing the same medium at different pH levels is shown.

these two media was 5.43 and 6.62, respectively. The next medium in the series at pH 5.28, containing two per cent sodium propionate, completely inhibited growth. The control medium adjusted to pH 3.5, by the addition of lactic acid, showed abundant growth which was expected, as it is a common procedure in the routine determination of yeast and mold counts to lower the pH of a medium by the addition of lactic acid to check bacterial growth. The results of a subsequent trial in which the range of pH varied between 6.96 and 5.85 are shown in table 1. *Hormodendrum cladosporioides* was used. A diminishing amount of growth may be noted as pH was decreased. Figure 3 shows representative plates from this trial. A few contaminants are noticeable, particularly on the medium at pH 6.18. If the plate is examined closely, two small colonies of *Hormodendrum cladosporioides* may be observed.

In this experiment, the addition of hydrogen ions to the propionate system, which would depress the ionization of propionate acid present, resulted in a gradual decrease in growth.

THE EFFECT ON MOLD GROWTH OF ADDING VARYING AMOUNTS OF SODIUM PROPIONATE TO A NUTRIENT MEDIUM CONTAINING A DEFINITE QUANTITY OF PROPIONIC ACID

Sodium propionate was added in varying amounts to potato dextrose medium containing 0.009 M propionic acid. Growth obtained on such media was compared with growth on a series of media containing the same amounts of salt but no acid, and also with a series of media containing different molar concentrations of propionic acid. In this case 0.009 M propionic acid was used as the concentration in the medium to which the salt was added because, in that concentration, growth of molds previously used was relatively abundant, yet it was near the critical concentration and any effect of the salt addition should be shown readily. Two molds were used, *Cladosporium sp.* and *Hormodendrum cladosporioides*. The data are shown in table 2.

It may be observed that growth was relatively abundant in the series of media containing only sodium propionate. In the medium containing only propionic acid, growth gradually diminished as the concentration was increased. However, when a combination of both propionic acid and sodium propionate was present growth also decreased as the amount of sodium propionate was increased. No growth was observed in the medium containing 0.009 M propionic acid plus 0.4 gm. of sodium propionate. Either of these compounds when present in the medium at the exclusion of the other did not result in complete inhibition of growth. Apparently the addition of the common propionate ion to the propionic acid system present in the medium resulted in an increase in the inhibitory power of the system. One effect of this would be an increase in the undissociated acid.

TABLE 2

Comparison of mold growth on media containing; (a) sodium propionate, (b) propionic acid, and (c) propionic acid plus sodium propionate

Composition of medium	pH of medium	Relative amount of growth of	
		Cladosporium sp. (6 days)	*Hormodendrum cladosporioides* (6 days)
Potato-dextrose medium (control)	5.80	7 +	7 +
" " " +0.1 gm. sodium propionate	6.10	6 +	6 +
" " " +0.2 gm. " "	6.30	5 +	6 +
" " " +0.3 gm. " "	6.38	5 +	6 +
" " " +0.4 gm. " "	6.50	5 +	6 +
0.009 M propionic acid in potato-dextrose medium +0.1 gm. sodium propionate	4.90	2 +	3 +
0.009 M " " " " " " +0.2 gm. " "	5.14	1 +	2 + (–)
0.009 M " " " " " " +0.3 gm. " "	5.30	1 +	1 +
0.009 M " " " " " " +0.4 gm. " "	5.42	–	–
0.009 M propionic acid in potato-dextrose medium	4.52	3 +	4 +
0.010 M " " " " " "	4.55	2 +	3 +
0.012 M " " " " " "	4.46	1 + (–)	1 + (–)
0.014 M " " " " " "	4.37	–	–

Note: Sodium propionate concentrations shown are per 100 ml. of medium.

DISCUSSION

A recapitulation of the results shown in tables 1 and 2 is presented in table 3. The equation for acid dissociation was applied to the data and the concentrations of undissociated propionic acid, propionate ions, and total propionate in the media are given, showing their relationship to the inhibition of *Hormodendrum cladosporioides*. No correlation between growth and concentration of total propionate or propionate ions is apparent. Increased inhibition of growth may be observed with a corresponding increase or decrease of pH. The undissociated acid is the only component for which a correlation with growth inhibition was obtained. Growth gradually diminished as the concentration of undissociated propionic acid increased. The critical concentration at which growth was completely inhibited was within the range 0.0105–0.0106 M. Within the range of these data, at least, hydrogen ions and propionate ions are of importance only insofar as they affect the concentration of undissociated acid.

TABLE 3

Effect of various components of the propionic acid dissociation system on mold growth

	Concentration of		pH	Growth of *H. cladosporioides*	Concentration of		
	Sodium propionate	Propionic acid			Undissociated acid (HA)	Propionate ion (A −)	Total propionate (S)
	moles per liter	*moles per liter*			*moles per liter*	*moles per liter*	*moles per liter*
From table 2	None		5.80	7 +			
	0.0104		6.10	6 +	0.0005	0.0099	0.0104
	0.0208		6.30	6 +	0.0007	0.0207	0.0208
	0.0312		6.38	6 +	0.0009	0.0303	0.0312
	0.0416		6.50	6 +	0.0009	0.0407	0.0416
From table 2		0.0090	4.52	3 +	0.0061	0.0029	0.0090
		0.0100	4.55	2 +	0.0066	0.0034	0.0100
		0.0120	4.46	1 +	0.0085	0.0035	0.0120
		0.0140	4.37	None	0.0105	0.0035	0.0140
From table 2	0.0104	0.0090	4.90	2 +	0.0091	0.0103	0.0194
	0.0208	0.0090	5.14	1 +	0.0101	0.0197	0.0298
	0.0312	0.0090	5.30	1 +	0.0106	0.0296	0.0402
	0.0416	0.0090	5.42	None	0.0108	0.0398	0.0506
From table 1	0.2080		6.96	6 +	0.0034	0.2046	0.2080
	0.2080		6.27*	1 +	0.0076	0.2004	0.2080
	0.2080		6.18*	1 + (−)	0.0093	0.1987	0.2080
	0.2080		6.11*	None	0.0109	0.1971	0.2080
	0.2080		5.85*	None	0.0190	0.1890	0.2080

* pH lowered by addition of lactic acid.

+ Indicates extent of growth.

CONCLUSION

From the data presented it is concluded that the undissociated propionic acid molecules play the major rôle in inhibiting the growth of molds used in this study.

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ANNOUNCEMENT

ANNUAL MEETING, IOWA STATE COLLEGE

June 18–20, 1946

REGISTRATION AND HOUSING

Registration headquarters will be in the Memorial Union, Iowa State College.

Housing facilities will be available in college dormitories and local hotels. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

PROJECTION EQUIPMENT

Lanterns will be available, upon request, for projection of standard and 2″ × 2″ slides. Projectors for 8 and 16 mm. movies also will be available by arrangement. Requests for projection equipment should be made at the time abstracts of papers are submitted to the respective section chairmen.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section committee meetings should contact Elmer Hansen. F. E. Nelson will assign rooms for meetings of committees of the Manufacturing Section.

SPECIAL MEETINGS

Rooms for a limited number of breakfasts, luncheons or dinners for special groups will be available at the Union. Shortage of help will not permit table service, so the groups will have to go through the cafeteria lines and thence to the reserved rooms. Requests for such reservations should be made to F. E. Nelson, Dairy Industry Department, Iowa State College, Ames, Iowa.

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LACTIC ACID, PYRUVIC ACID, AMINO ACIDS, ACETONE BODIES, OXYGEN, CARBON DIOXIDE, AND HEMOGLOBIN IN ARTERIAL AND MAMMARY VENOUS BLOODS OF COWS UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

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The study of the blood precursors of milk, which received its early impetus by the technique of Kaufmann and Magne (14), and in which interest has been heightened in more recent years by Graham's (11, 13) techniques of obtaining arterial blood, has contributed greatly to our knowledge of the metabolism of the mammary gland. It is only natural that such techniques should continue to be refined and that with such improvements the work should be expanded and some of the earlier interpretations altered in the light of more recent studies. One such refinement was the detection by Shaw and Petersen (28) of the relationship between excitation and changes in the concentration of hemoglobin in the blood traversing the mammary gland, which led to the finding by Powell and Shaw (19) that the reported uptake of blood lactic acid by the active gland was only an apparent utilization caused by agitation of the animal, with a resultant rapid increase in the lactic acid in the arterial blood and a temporary disproportion between the arterial and venous concentration of lactic acid. One of the purposes of this work was to obtain more conclusive data as to whether blood lactic acid is taken up by the active gland. A companion substance, pyruvic acid, was also included in this study.

In view of the conflicting reports on arteriovenous hemoglobin changes and the respiratory quotient of the mammary gland, these studies were also extended. In addition a series of arteriovenous experiments were conducted on cows in which the per cent of milk fat and the lower fatty acids of milk had been decreased by feeding fish oils.

EXPERIMENTAL PROCEDURE

As both lactic acid and pyruvic acid increases rapidly with excitation, numerous arteriovenous studies were made of these two substances on anes-

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thetized lactating and nonlactating cows, fasted cows, and cows fed cod-liver oil. The respiratory quotients of mammary glands of normal-lactating, fasted, and nonlactating-nonpregnant cows were also determined on cows under nembutal anesthesia. The respiratory quotients were also obtained on the mammary glands of both nonanesthetized and anesthetized cows in which the per cent of fat in milk had been depressed by feeding fish oil.

Amino acid determinations were made on arterial and mammary venous bloods of anesthetized cows before and after depressing the per cent of milk fat by the feeding of red-fish oil. In the studies on the effect of fish oils, blood samples were not taken in any case until a large decrease in the per cent of milk fat had been observed. Acetone bodies were determined on arterial and mammary venous bloods of nonanesthetized cows before and after feeding cod-liver oil. Hemoglobin determinations were made on all blood samples.

Arterial bloods were obtained from either the internal iliac or prepubic arteries by rectal puncture. Mammary venous bloods were obtained from the subcutaneous abdominal veins. Ethyl chloride was used to anesthetize the skin in the region of the venipuncture on nonanesthetized cows. After repeated venipunctures cows are subject to more agitation. Local ethyl chloride anesthesia prolonged the period that the animals remained unagitated. Once the animals reached the stage where they anticipated the venipuncture, local anesthesia only served to agitate the animals further. Blood samples drawn from highly agitated cows were usually discarded. Determinations were made on a few, however, to demonstrate the effect of excitation upon arteriovenous hemoglobin differences.

In anesthetizing the animals the nembutal was injected intravenously while the animal was standing in order to avoid undue disturbance. From three to five grams were injected within a period of 30 seconds, the dosage varying with the size and condition of the cow. The animal was allowed to fall in a well-bedded stall and kept on its sternum by the use of bags filled with sawdust. The head was always kept in a low position to allow for the draining of saliva. During the first hour approximately one gram of nembutal was injected intravenously at 20-minute intervals to maintain anesthesia. The respiration rate was taken at frequent intervals to aid in maintaining a moderate anesthesia. After the first hour nembutal injections were made at less frequent intervals. The cows usually remained lying down for several hours after regaining consciousness. In a few cases blood samples were obtained after the cows had partially recovered from anesthesia but were still not reactive to the needle. After partial recovery from the effects of the anesthesia the cows were always rolled over on the opposite side to improve circulation, still maintaining the fore part of the animal in an upright position. When such procedure was followed, no serious after affects were observed. A light feeding of grain was always given a few hours before anesthetizing the animals in order to avoid any alterations in the metabolism of the mammary gland due to fasting.

Early in the course of the work it was observed that large fluctuations occurred in both the arteriovenous differences and in the absolute values when blood samples were taken too soon after anesthetization. This was particularly true of oxygen, carbon dioxide, hemoglobin, lactic acid and pyruvic acid. For this reason blood samples were usually not taken until 15 to 20 minutes after anesthetization and in most cases a longer period of time was allowed to elapse. In addition the samples were usually drawn after the animal had been very quiet and the rate of respiration had been steady and normal for from 10 to 15 minutes or more. As there is a tendency for the arteriovenous differences to be similar over relatively short periods, rather lengthy periods of time were allowed to elapse between samples in order to obtain a more representative picture of the metabolism of the gland.

Bloods for lactic acid were taken in oxalated flasks containing sodium fluoride and chilled in ice water. Bloods for amino acids and hemoglobin were taken in oxalated vessels. Bloods for oxygen and carbon dioxide were drawn under oil in vessels containing heparin. Bloods for pyruvic acid were drawn last in clean flasks and immediately pipetted into cold trichloracetic acid without the use of preservative or anticoagulant. When consecutive arteriovenous samples were being drawn, the bloods for pyruvic acid were always pipetted into trichloracetic acid before the next series of blood samples were taken. Arterial and mammary venous bloods for each substance to be determined were always drawn simultaneously.

The chemical methods used were as follows: acetone bodies, Barnes and Wick (3); glucose, Somogyi's (32) modification of the Shaffer and Somogyi method; lactic acid, Miller and Muntz (18) as modified by Koenemann (17) and Barker and Summerson (2); pyruvic acid, Friedemann and Haugen (7); hemoglobin, Evelyn and Malloy (5); amino acids, Frame *et al.* (6) and Russell (22); oxygen and carbon dioxide, Van Slyke and Neill (33) and the Reichert-Meissl value of fat, Official and Tentative Methods of Analysis (1).

RESULTS

The Respiratory Quotient of the Mammary Glands of Normal Lactating, Lactating Fasted, and Nonlactating, Nonpregnant Cows Under Nembutal Anesthesia

A total of 22 respiratory quotients were obtained from eight normal lactating cows under anesthesia. The mean respiratory quotient was 1.27 which confirms the reports of Graham *et al.* (9) and Reineke *et al.* (20) on goats and is in accord with the findings of Shaw and Petersen (29) on cows in which there were no arteriovenous hemoglobin differences. The low respiratory quotient on both fasting and nonlactating cows is also in accord with previous reports (20, 31) although more data are needed to establish these relationships with certainty.

The Respiratory Quotient of Lactating Mammary Glands of Cows Fed Fish Oil

In view of the reports that the respiratory quotient of the mammary gland of the fasted cow is less than unity and the suggestion by Reineke

TABLE 1

Arterial and mammary venous blood oxygen and carbon dioxide on cows under nembutal anesthesia

Experiment No.	Minutes under anesthesia	Respiration rate	Per cent hemoglobin difference	O_2 difference, vol.%	CO_2 difference, vol.%	R.Q.
Normal lactating cows						
D.E. 242	20	Normal	−0.31	+3.58	−5.37	1.50
	90	Normal	−0.50	+3.62	−4.54	1.25
	110	Normal	+0.49	+2.77	−2.90	1.05
D.E. 244	30	Normal	0.00	+2.12	−2.52	1.19
	95	Normal	0.00	+3.20	−3.49	1.09
D.E. 245	10	Normal	0.00	+3.08	−2.84	0.92
	85	Normal	0.00	+2.84	−3.38	1.19
	120	Normal	0.00	+3.21	−4.17	1.30
	122	Normal	0.00	+3.11	−2.87	0.92
D.E. 246	107	Normal	+0.64	+3.86	−4.39	1.14
D.E. 248	43	Normal	−1.71	+2.90	−2.25	0.78
	110	Normal	0.00	+2.73	−3.89	1.43
	320*	Normal	0.00	+3.88	−3.85	0.99
D.E. 249	28	Normal	0.00	+2.36	−4.72	2.00
	31	Normal	−0.98	+2.79	−3.76	1.35
	43	Normal	0.00	+1.76	−3.72	2.11
D.E. 250	30	Normal	−0.95	+3.30	−3.80	1.15
	57	Normal	−0.73	+2.64	−3.52	1.33
	60	Normal	0.00	+3.40	−5.26	1.55
D.E. 251	91	Rapid	−1.31	+3.83	−5.55	1.45
	102	Rapid	−0.76	+4.00	−4.91	1.23
	182*	Normal	−0.81	+5.85	−5.85	1.00
Average			0.48	+3.22	−3.98	1.27
Lactating cow fasted 40 hours						
D.E. 273	40	Normal	+0.84	+2.23	−1.34	0.60
	55	Normal	0.00	+2.19	−2.70	1.23
	58	Normal	0.00	+3.28	−2.62	0.80
Average			0.28	+2.57	−2.22	0.88
Nonlactating-nonpregnant cow						
D.E. 272	50	Normal	−1.65	+2.28	−0.94	0.41
	53	Normal	0.00	+4.37	−3.07	0.70
	58	Normal	0.00	+3.82	−2.76	0.72
Average			0.55	+3.49	−2.26	0.61

* Conscious but not reactive to needle.

et al. (20) that the low quotient is due to a decrease in the synthesis of the lower fatty acids of milk from carbohydrate, a study was made to determine whether the relationship between the low quotient and the decrease in the lower fatty acids on fasting was real or fortuitous. As the feeding of cod-liver oil also decreases the short-chain fatty acids of milk fat markedly, a low respiratory quotient should result if the low quotient on lactating glands of fasted cows is to be accepted as evidence that such acids are synthesized from carbohydrate.

TABLE 2

Arterial and mammary venous blood oxygen and carbon dioxide on cows fed fish oil

Experiment No.	Days fed fish oil	Milk fat, per cent	Minutes under anesthesia	Condition of cow	Per cent hemoglobin difference	O_2 difference, vol.%	CO_2 difference, vol.%	R.Q.
	Cows fed cod-liver oil							
D.E. 50	0	3.0						
	43	1.8		Quiet	−0.76	+4.63	−4.42	0.95
	Two minutes between samples			Quiet	−0.76	+3.97	−3.69	0.93
D.E. 51	0	2.8						
	18	2.1		Quiet	0.00	+3.70	−2.62	0.71
D.E. 53	0	3.5						
	37	1.5		Quiet	0.00	+3.95	−2.76	0.70
	Two minutes between samples			Quiet	0.00	+4.16	−2.56	0.62
D. E.54	0	4.4						
	24	3.2		Quiet	0.00	+4.25	−5.76	1.36
	Two minutes between samples			Quiet	−0.94	+4.38	−6.95	1.59
D.E 55	0	6.1						
	15	4.5		Agitated	+2.18	+3.80	−8.36	2.20
D.E. 56	0	3.2						
	17	1.9		Quiet	0.00	+6.25	−7.13	1.14
	Two minutes between samples			Quiet	−0.68	+6.03	−8.64	1.43
	Two minutes between samples			Quiet	0.00	+5.10	−6.77	1.33
	23	1.9		Agitated	+5.75	+4.42	−6.23	1.41
	24	2.0		Agitated	+1.44	+4.62	−5.67	1.23
D.E. 57	0	4.2			0.96	+4.56	−5.50	1.20
	17	3.1		Agitated	+1.44	+3.58	−2.27	0.63
Average of nonanesthetized cows					1.00	+4.48	−5.26	1.16
	Cows fed red-fish oil—respiration normal in all cases							
D.E. 251	0	6.2						
	23	3.2	71		0.00	+1.18	−1.28	1.08
			228*		0.00	+2.74	−3.38	1.23
D.E. 251	32	3.0	75		0.00	+3.42	−5.28	1.54
			127		0.44	+1.98	−3.52	1.78
D.E. 250	0	5.5						
D.E. 250	21	3.0	76		0.00	+3.08	−3.18	1.03
D.E. 250	26	3.2	57		+1.53	+3.05	−3.87	1.27
	....		77		+0.76	+3.10	−3.79	1.22
Average of anesthetized cows					0.38	+2.65	−3.47	1.31

* Conscious but not reactive to needle.

Eight cows were fed either cod-liver oil or red-fish oil until the per cent of milk fat had been decreased markedly. Red-fish oil was used because it has been found to be at least as potent as cod-liver oil in decreasing the per cent of milk fat and the lower fatty acids. From 300 to 450 ml. of fish oil per day were fed over extended periods of time to obtain the maximum effect.

In the initial trials on nonanesthetized cows, a low quotient was obtained and it was believed that the similarity to fasted cows prevailed. More detailed analyses, however, failed to confirm these results. As noted in table 2, the average respiratory quotient of fifteen experiments on six nonanesthetized cows was 1.16. In addition, seven such calculations were made from experiments on two cows under nembutal anesthesia in which the per cent of milk fat had been depressed by the feeding of red-fish oil. The mean respiratory quotient was 1.31. In a total of 22 such experiments, the respiratory quotients on the active glands of cows fed fish oil exceeded unity in 15 cases with a mean value of 1.21. As a check on the relationship of the decrease in the per cent of milk fat and the lower acids, Reichert-Meissl values were determined on the milk fat of two cows before and after the depression of the per cent of fat by feeding fish oil. D.E. 53 exhibited a decrease from 38.56 to 25.09 and D.E. 56 a decrease from 36.50 to 22.84.

Arteriovenous Studies on Lactic Acid and Pyruvic Acid

Since excitation has such a marked influence on the apparent uptake of lactic acid by the active mammary gland it seemed advisable to study this matter further by the use of anesthetized animals. Pyruvic acid determinations were also included in these studies. Cows in various physiological conditions were studied, since it was entirely possible that under certain conditions the metabolism of the gland might be altered in such a way as to effect changes in the metabolism of either pyruvic acid or lactic acid or both. As noted in table 3, 22 analyses on six normal lactating cows under anesthesia showed an arteriovenous difference in lactic acid of only + 0.18 mg. per cent. A total of 26 analyses on eight normal lactating cows under anesthesia showed an arteriovenous difference of only + 0.047 mg. per cent pyruvic acid. It is apparent from these data that neither blood lactic acid nor pyruvic acid play a significant rôle in the elaboration of milk. Likewise, the arteriovenous differences in these two substances on fasted, nonlactating, and fish-oil-fed cows were insignificant and did not suggest any alteration in the metabolism of either lactic acid or pyruvic acid. The only cases in which a significant amount of either substance was taken up by the gland was shortly after anesthetization, when the arterial blood was still apparently slightly above normal due to the excitation of the animal during the initial injection of nembutal, although even in these cases the largest positive difference was only 1.10 mg. per cent lactic acid and 0.424 mg. per cent pyruvic acid.

TABLE 3

Arterial and mammary venous blood hemoglobin, lactic acid, and pyruvic acid on cows under anesthesia

Cow No.	Minutes under anesthesia	Hemoglobin			Lactic acid			Pyruvic acid		
		Arterial	Venous	Per cent difference	Arterial	Venous	Difference	Arterial	Venous	Difference
		Normal lactating cows								
		%	%			*mg.%*	*mg.%*	*mg.%*	*mg.%*	*mg.%*
D.E. 242	20	13.00	13.04	−0.31				0.708	0.542	+0.166
	90	12.83	12.89	−0.50				0.528	0.454	+0.074
	110	14.25	14.18	+0.49				0.463	0.426	+0.037
D.E. 243	5	11.83	12.10	−2.28				1.104	0.680	+0.424
	85	13.75	13.75	0.00				0.442	0.442	0.000
	105	14.04	13.97	+0.50				0.358	0.434	−0.076
D.E. 244	12	10.74	10.74	0.00	6.08	5.87	+0.21			
	30	10.81	10.81	0.00	3.29	3.03	+0.26	0.448	0.310	+0.138
	95	10.57	10.57	0.00	2.02	2.21	−0.19	0.296	0.318	−0.022
D.E. 245	10	10.41	10.41	0.00	4.45	3.66	+0.79	0.596	0.410	+0.186
	85	11.07	11.07	0.00	1.70	1.51	+0.19	0.318	0.230	+0.088
	120	12.00	12.00	0.00	2.27	2.03	+0.24	0.318	0.282	+0.036
D.E. 247	38	12.17	12.17	0.00	6.25	6.07	+0.18	0.466	0.410	+0.056
	99	12.09	12.09	0.00	8.27	9.24	−0.97	0.445	0.562	−0.117
	147	12.83	12.83	0.00	7.87	8.78	−0.91	0.484	0.664	−0.180
D.E. 248	43	10.48	10.66	−1.71	4.07	3.68	+0.39	0.390	0.310	+0.080
	110	10.74	10.74	0.00	2.52	2.34	+0.18	0.326	0.298	+0.028
	320*	11.66	11.66	0.00	4.20	3.24	+0.96	0.410	0.330	+0.080
	28	9.32	9.32	0.00	2.60	2.37	+0.23	0.290	0.264	+0.026
	31	9.23	9.32	−0.98	2.21	2.18	+0.03	0.306	0.264	+0.042
	43	9.23	9.23	0.00	2.15	2.23	−0.08	0.290	0.274	+0.016
D.E. 250	30	9.39	9.48	−0.95	4 66	3.56	+1.10	0.314	0.258	+0.056
	57	9.63	9.70	−0.73	3.78	3.14	+0.64	0.290	0.258	+0.032
	60	9.78	9.78	0.00	3.28	2.97	−0.31	0.322	0.294	+0.028
D.E. 251	21	9.85	10.09	−2.44	4.66	4.10	+0.56	0.458	0.378	+0.080
	91	10.68	10.82	−0.14	2.72	2.61	+0.11	0.306	0.322	+0.016
	102	10.57	10.65	−0.76	2.73	3.37	−0.64	0.322	0.370	−0.048
	182*	9.93	10.01	−0.81	3.10	2.83	+0.27			
Average				0.45	3.86	3.68	+0.18	0.423	0.376	+0.047
		Lactating cows fed red-fish oil								
D.E. 251	51	9.40	9.43	−0.32	1.85	1.83	+0.02	0.501	0.501	0.000
	71	10.17	10.17	0.00	1.75	2.38	−0.63	0.593	0.536	+0.057
	228	10.01	10.01	0.00	1.97	3.93	−1.96	0.450	0.560	−0.110
D.E. 250	43	10.49	10.49	0.00	3.74	3.75	−0.01	0.676	0.596	+0.080
	76	10.49	10.49	0.00	2.07	2.31	−0.24	0.556	0.466	+0.090
	85	10.49	10.49	0.00	2.18	2.19	−0.01	0.516	0.460	+0.056
D.E. 250	57	10.49	10.33	+1.53	1.69	1.69	0.00	0.373	0.298	+0.075
	77	10.49	10.41	+0.76	1.56	1.54	+0.02	0.298	0.280	+0.018
Average				0.31	2.10	2.45	−0.35	0.495	0.462	+0.033
		Lactating cow fasted 40 hours								
D.E. 273	40	10.74	10.65	−0.84	3.77	3.67	+0.10	0.370	0.306	−0.064
	55	10.49	10.49	0.00	3.33	3.36	−0.03	0.374	0.317	−0.057
	58	10.49	10.49	0.00	3.49	3.26	+0.23	0.374	0.354	−0.020
Average				0.28	3.53	3.43	+0.10	0.372	0.326	−0.047
		Nonlactating-nonpregnant cow								
D.E. 272	50	12.71	12.92	−1.65	2.76	3.20	−0.44	0.342	0.426	−0.084
	53	12.71	12.71	0.00	2.33	2.75	−0.42	0.371	0.442	−0.071
	58	12.38	12.38	0.00	2.54	2.75	−0.21			. ..
Average	.	. . .		0.55	2.54	2.90	−0.36	0.357	0.434	−0.078

* Conscious but not reactive to needle.

Arteriovenous Studies on Acetone Bodies on Cows Before and After Feeding Fish Oil

Because the active mammary gland was found to utilize blood β-hydroxybutyric acid and not acetoacetic acid (25) the former substance appeared to be a possible precursor of the lower fatty acids of milk fat. Initial trials, which were made before the influence of the concentration of β-hydroxybutyric acid on its utilization was understood (23), appeared to confirm these suspicions. To avoid any possible misintrepretations due to the individuality of the animal, arteriovenous determinations were made on the total blood acetone bodies before and after the feeding of cod-liver oil. The cows were not anesthetized, as previous work had shown that excitation did not exert any significant influence on the arteriovenous difference (25). The data presented in table 4 do not indicate that the utilization of β-hydroxybutyric acid by the lactating mammary gland is altered by the feeding of cod-liver oil. Although the mean arteriovenous difference is greater in the cows fed cod-liver oil, this difference is undoubtedly due to a higher than normal concentration of acetone bodies in the arterial blood of three of the cows since the utilization is known to be greater at higher than normal concentrations (23, 30).

TABLE 4

Arterial and mammary venous blood acetone bodies on nonanesthetized cows before and after feeding cod-liver oil

Cow No.	Per cent hemoglobin difference	Acetone bodies (as acetone)		
		Arterial	Venous	Difference
Normal cows				
		mg. %	*mg. %*	*mg. %*
D.E. 54	0.83	2.93	2.01	+0.92
D.E. 55	1.32	1.83	1.14	+0.79
D.E. 56	0.00	3.13	2.31	+0.82
D.E. 57	0.89	2.45	1.40	+1.05
Average	0.76	2.59	1.72	+0.90
Cod-liver-oil-fed cows				
D.E. 54	0.00	2.88	1.64	+1.24
D.E. 55	0.00	6.29	4.04	+2.25
D.E. 56	0.00	7.60	5.35	+2.25
	−0.68	7.75	5.43	+2.32
D.E. 56	−5.75	2.27	1.33	−0.94
	−1.44	3.06	2.29	+0.77
D.E. 57	+1.44	2.29	1.22	+1.07
Average	1.33	4.59	3.04	+1.55

Arteriovenous Studies on Amino Acids on Anesthetized Cows Before and After Feeding Red-fish Oil

The utilization of amino acids by the gland of the normal cow (4, 12, 27), the formation of urea (10), together with the presence of arginase in the gland (26), and the lack of utilization of amino acids reported for the fasted cow (21, 31), suggested that the free blood amino acids should be given consideration as possible precursors of the lower fatty acids. Calculations on the basis of the calcium and amino acid uptake by the gland and the total calcium and lower fatty acids in milk had shown that the lower fatty acids could be accounted for by the amount of free amino acids taken up by the gland (31).

TABLE 5

Arterial and mammary venous blood amino acids on a nonlactating cow and on lactating cows before and after feeding red-fish oil

Cow No.	Per cent hemoglobin difference	Amino acids		
		Arterial	Venous	Difference
		Normal cows		
		mg. %	*mg. %*	*mg. %*
D.E. 250	-0.95	5.86	5.19	+0.67
	-0.73	5.38	5.05	+0.33
	0.00	5.22	4.66	+0.56
D.E. 251	-2.44	6.17	5.71	+0.46
	-1.31	5.65	5.32	+0.33
	-0.76	5.77	5.40	+0.37
	-0.81	5.50	4.95	+0.55
Average	-1.00	5.65	5.18	+0.47
		Red-fish-oil-fed cows		
D.E. 250	0.00	5.64	5.19	+0.45
	0.00	5.64	5.31	+0.33
	0.00	5.58	5.12	+0.46
D.E. 251	-0.32	5.59	5.29	+0.30
	0.00	5.58	5.09	+0.49
	0.00	5.54	5.33	+0.21
Average	-0.05	5.60	5.22	+0.37
		Nonlactating-nonpregnant cow		
D.E. 272	-1.65	5.67	5.72	-0.05
	0.00	5.67	5.72	-0.05
	0.00	5.56	5.56	0.00
Average		5.63	5.67	0.03

Accordingly a series of amino acid determinations were made on the arterial and mammary venous bloods of cows before and after the per cent of milk fat had been depressed by the feeding of red-fish oil.

These animals were anesthetized because studies on substances known to be affected by excitation were also being made. As noted in table 5, the feeding of red-fish oil did not materially alter the utilization of amino acids by the active mammary gland, the mean arteriovenous difference before and after feeding fish oil being 0.47 mg. per cent and 0.37 mg. per cent, respectively. The decrease in the lower fatty acids effected by feeding fish oil cannot, therefore, be considered as due to a failure of the gland to take up amino acids from the blood. *Three arteriovenous values obtained on a dry* cow provide a good check on the technique and demonstrate that the inactive gland does not take up blood amino acids.

ARTERIOVENOUS HEMOGLOBIN DIFFERENCES

It was first shown by Shaw and Petersen (28) that marked agitation resulted in large changes in the concentration of the blood traversing the mammary gland. Such differences did not occur in the nonexcited cow and therefore the arteriovenous hemoglobin differences could be used as a criterion of the degree of excitation provoked in the animal. Bloods drawn from cows under anesthesia should provide an excellent opportunity to study this matter further. With moderate anesthesia the arteriovenous differences in hemoglobin might be expected to resemble those of the nonexcited, nonanesthetized animal. Arterial and mammary venous hemoglobin values are presented in table 3 in some detail. It will be observed that out of a total of 42 arteriovenous hemoglobin determinations all but five exhibited less than 1.0 per cent hemoglobin difference and in 30 of the 42 experiments the difference was not over 0.5 percent. Further, in all five of the experiments exhibiting over 1.0 per cent hemoglobin difference the bloods were the first of a series of samples in which none of the later samples exhibited this degree of change. In these five cases the animals had either been under anesthesia but a short time or the respiration had been steady for but a short period. The average arteriovenous hemoglobin difference for the 42 experiments was 0.40 per cent. This compares favorably with the average of 0.64 reported by Shaw and Petersen (28) on nonexcited cows and may be constrasted with the much larger hemoglobin per cent difference of 5.70 observed on excited cows (28).

DISCUSSION

The failure of the respiratory quotients of the mammary glands of cows fed fish oils to parallel that reported for fasted cows leaves the interpretation of the low respiratory quotient of the latter in doubt. One fact which has troubled the writer for some time is that there is a considerable quantity of the lower fatty acids being synthesized after several days of fasting and yet the quotient is apparently below unity. If the respiratory quotient of the gland can be used safely as an indication of the type of metabolism taking place in the gland, it must mean that considerable oxidation of fat is taking place in the fasted cow. If β-hydroxybutyric acid is oxidized in the gland for energy purposes, as appears to be the case, it would help to account for a quotient below unity in the fasted cow. However, the complete oxidation of this substance would result in a quotient of 0.89 and but very little synthesis of fat from carbohydrate would be needed to elevate the quotient to unity or above. The report that several days of fasting are required to produce a quotient below unity (20) is also not in accord with the suggestion that the low quotient demonstrates that the lower fatty acids are synthesized from carbohydrate, as the major decrease in these acids occurs within 24 to 48 hours (15) of fasting. However, the data on the fasted cow are very limited and further speculation is probably unwarranted until more information is obtained on both fasted and fish-oil-fed animals.

The low respiratory quotient of the inactive mammary gland is also difficult to evaluate. If it were due to the oxidation of fat it would appear that the gland of the nonlactating cow should utilize the readily oxidizable β-hydroxybutric acid. Such does not appear to be the case, however (25). It is significant that the low quotients on the mammary gland have been reported only in those cases in which the gland has been relatively inactive, as in the case of the gland of the nonlactating cow and the cow fasted for several days. In the latter case milk production drops to a very low level within a matter of two to three days. A possible explanation is that the lymph of the inactive gland may be carrying away a larger proportionate amount of carbon dioxide resulting in an apparent rather than a real respiratory quotient below unity. This would also explain the failure to obtain consistently low values on the glands of cows in which the lower acids have been depressed by the feeding of fish oil, as milk production is not materially affected by the feeding of these oils.

The nonutilization of amino acids reported for the active gland of the fasted animal may also be due to the precipitous drop in milk secretion in which the usual functions of these acids may be amply provided for by amino acids taken up by the gland as protein. At least the apparent normal utilization of blood amino acids by the active gland of the fish-oil-fed cow does not suggest any relationship between the amino acid utilization and the lower fatty acids.

The failure of cod-liver oil to effect a decrease in the utilization of acetone bodies by the gland, together with the finding that the fasted-ketotic cow exhibits an increased utilization of β-hydroxybutyric acid with a decrease in the lower fatty acids of milk fat, appears to rule out the possibility of β-hydroxybutyric acid acting as a precursor of the lower acids and favors our alternative suggestion that this substance is used by the gland for energy purposes (25).

An increase of over 100 per cent occurs in the utilization of β-hydroxybutyric acid by the gland in ketonemia (23, 30) and since it appears to be utilized for energy purposes offers some excellent possibilities in studying the energy metabolism of the gland. The fact that practically all of the oxidation by the gland in ketonemia (23, 30) can be accounted for by the uptake of β-hydroxybutyric acid indicates that a shift must take place from the oxidation of both β-hydroxybutyric acid and other substances to the sole oxidation of β-hydroxybutyric acid. This assumes that β-hydroxybutyric acid is used for energy purposes, an assumption that appears valid on the basis of our present knowledge of the metabolism of this substance. Information as to the substances involved in such a shift would be invaluable to a more complete understanding of the metabolism of the gland. The possibility that one of the substances may be fatty acids other than β-hydroxybutyric acid should be explored further even though the short-chain fatty acids of nonfasted ketotic cows did not appear to be appreciably low (31).

The data on lactic acid values of cows under anesthesia amply confirm our previous observations that the arteriovenous change in the concentration of this substance is insignificant in nonexcited cows (19). The mean arteriovenous difference in these studies of + 0.18 mg. per cent is in sharp contrast with the earlier reports by Graham (8) and Shaw, Boyd and Petersen (24) of a mean utilization of 16.70 and 3.64 mg. per cent, respectively, on goats and cows, but is in substantial agreement with the arteriovenous value of + 0.52 mg. per cent reported by Powell and Shaw (19) for unagitated cows, a value shown to be statistically insignificant.

The relatively insignificant arteriovenous difference in pyruvic acid of + 0.047 mg. per cent obtained from 26 experiments on lactating cows under anesthesia was somewhat surprising in view of the utilization of this substance found by Knodt and Petersen (16) in incubated tissue. However, it does demonstrate that blood pyruvic acid does not play a significant rôle in the metabolism of the gland of the cow under normal conditions.

The lactic acid and pyruvic acid values obtained on fasted, nonlactating, and fish-oil-fed cows do not show any significant difference from the normal cows. The relatively low mean arterial value of 3.86 mg. per cent lactic acid on normal cows under anesthesia is considerably less than the value of 7.29 mg. per cent found on nonexcited, nonanesthetized cows (19) and suggests that even in those cases in which the cow does not exhibit excitation there is significant disturbance to effect an increase in blood lactic acid.

There is some reason to believe that a rather close relationship exists between rumen digestion and the synthesis of the lower fatty acids of milk fat. It is entirely possible that some of the effects of fasting and fish oils on the synthesis of milk fat are related to an alteration in rumen digestion. It appeared probable that some of the end products of bacterial fermentation would contribute to the concentration of certain blood substances, in which case any marked change in rumen digestion might be expected to result in a change in the level of such substances in the blood. Two such are lactic acid and pyruvic acid. Fasting did not appear to influence the blood concentration of either substance. Also, the feeding of red-fish oil did not appear to influence the blood pyruvic acid. However, the values for blood lactic acid are consistently lower for the cows on red-fish oil than for the normal cows, the arterial concentration being 2.10 mg. per cent as compared to 3.86 mg. per cent for cows fed a normal diet. However, more data are needed to establish the validity of this observation. Certainly the feeding of cod-liver oil did not decrease the blood acetone bodies; substances for which unpublished work in this laboratory has indicated rumen precursors.

The mean difference in blood hemoglobin concentration of 0.40 per cent on 42 anesthetized cows is in sharp contrast with the value of 5.70 per cent obtained by Shaw and Petersen (28) on 23 excited cows and in substantial agreement with the value of 0.64 on nonexcited cows. As would be expected, the arteriovenous changes on anesthetized animals show greater uniformity.

The question may be raised as to whether nembutal anesthesia interferes with milk secretion. Reineke *et al.* (20) concluded that milk secretion was not diminished by nembutal. The following data were obtained on three cows which had been under nembutal anesthesia for one to two hours and in which the animals recovered sufficiently to be milked at the usual time.

		D.E. 251, lbs. milk	*D.E. 250, lbs. milk*	*D.E. 249, lbs. milk*
Day before anesthesia	A.M.	10.3	13.4	6.2
	P.M.	9.2	9.7	4.4
Day of anesthesia	A.M.	11.6	13.7	6.4
	P.M.	8.5	8.9	4.4
Day after anesthesia	A.M.	10.0	13.0	6.1
	P.M.	9.9	8.5	4.3

There was very little diminution in the rate of milk secretion in these experiments. In each case the cow was put under anesthesia after the morning milking. As the cows were fed lightly on the morning of the experiment, some decrease might have been expected the following day. More conclusive information could have been obtained by the use of oxytocin injections at short intervals, but the data do indicate that no large decrease occurred in milk production.

SUMMARY AND CONCLUSIONS

1. The respiratory quotient of the active mammary glands of both normal and fish-oil-fed cows under nembutal anesthesia exceeded unity with mean values of 1.27 and 1.31 respectively. In experiments with nonanesthetized cows in which the per cent of fat and the low fatty acids of milk fat had been depressed by feeding fish oil, the respiratory quotient exceeded unity in 9 of the 15 experiments with a mean quotient of 1.16. On the basis of these data the suggestion that a metabolic relationship exists between the respiratory quotient of the gland and the synthesis of the lower fatty acids of milk is still in doubt.

2. Numerous experiments on cows under anesthesia confirm the earlier report (19) that the mammary gland does not utilize a significant amount of blood lactic acid. The mean arteriovenous value for 22 experiments was + 0.18 mg. per cent.

3. The mean arteriovenous difference for pyruvic acid in 26 experiments

was + 0.047. Consequently, blood pyruvic acid appears to be of little significance in the metabolism of the active mamary gland.

4. The arteriovenous differences of lactic acid and pyruvic acid were not altered significantly on fish-oil-fed, fasted, or nonlactating cows from that observed on normal lactating cows.

5. Neither the utilization of amino acids nor acetone bodies were altered significantly by depressing the per cent of milk fat and the lower fatty acids by feeding fish oils.

6. Arteriovenous hemoglobin studies on anesthetized cows confirm earlier observations that little or no arteriovenous change occurs in unagitated cows. The per cent hemoglobin difference for 42 experiments on anesthetized cows was 0.40 and exceeded one per cent in only 5 cases.

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EFFECT OF WARM WEATHER ON GRAZING PERFORMANCE OF MILKING COWS

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Grazing habits of dairy cattle are of direct concern to dairymen whenever they help explain increases or decreases in milk production. Published reports on activities of dairy cattle while on pasture are relatively few and none has been found that reported the effect that changes in atmospheric temperatures have on grazing habits. Hodgson (3) in 1933 reported that dairy cows at Puyallup, Washington, spent from 6½ to 7 daylight hours grazing under a rotational pasture system and from 7 to 7½ hours on a continuous grazing plan. No records were kept of their grazing habits at night.

A more detailed study by Atkeson, Shaw, and Cave (1) showed that Kansas milking cows during 11.7 daylight hours grazed 5.6 hours while on good pasture; 6.5 hours on fair pasture; and 7.3 hours on poor pasture. The cows lay down an average of four times on good pasture and only two times on fair or poor pasture. Records taken on 56 dry cows and heifers while on excellent Balbo rye pasture day and night during April showed an average of 7 hours grazing; 4 hours standing or walking; and 13 hours lying down. During the 14-hour daylight period the animals grazed 40 per cent of the time; while only 16 per cent of the 10-hour night period was spent grazing. They lay down 35 per cent of the daylight period and 80 per cent of the night. Grazing seemed to be spaced into four primary periods.

Records on beef cattle (4) taken in New York during July, August, and September averaged 7 hours and 32 minutes of grazing time during the 24 hours of the day. Of this, 4 hours and 23 minutes took place between 7 A.M. and 7 P.M., and 3 hours and 9 minutes between 7 P.M. and 7 A.M.

The present study, while similar in some respects to those reviewed, had two primary objectives: First, to determine changes in body temperature and pulse and respiration rates of cows on pasture during relatively warm days and second, how the warm weather affected the grazing habits of milking cows.

EXPERIMENTAL

During a period of three days, September 4, 7, and 8, 1945, observations were made on 6 milking cows, 3 Jerseys and 3 Holsteins, in an effort to determine changes in their body temperatures, pulse rates, and respiration rates during the daytime and while they were on pasture. Rectal temperatures, pulse counts from coccygeal arteries of the tail, and respiration counts from flank movements were made during five periods of the day, *i.e.*, just before cows entered pasture at 5:45 A.M.; when they entered shade (averaged 9:20

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A.M.); one hour after entering shade; at 2:00 P.M.; and at 3:00 P.M. The 5:45 A.M. observation was taken in the barn immediately after the morning milking; while the 3:00 P.M. reading was made after cows were put into the barn prior to feeding, washing, and the evening milking. All other observations took place in the pasture. This was made possible by using cows relatively tame and by tying them with halters during the time observations were made.

Observations relative to grazing performance during five 24-hour periods constituted the second phase of the experiment. The same six milking cows, with the exception of one substitute Holstein cow, were used. Observations were made on September 10 to 14, inclusive. The days selected proved to be fortunate choices, for average daily air temperatures in the shade (table 2) varied from 72° to 86° F. during the daytime and from 62° to 81° F. for the nights. These wide variations made it possible to observe changes in grazing habits caused by air-temperature changes.

By the use of a sling psychrometer wet- and dry-bulb, atmospheric temperature determinations were made throughout the period of the experiment. This was done in order to determine changes in atmospheric temperature and humidity from day to day and during the course of each day.

Cows on the experiment had access to relatively good permanent pasture, which consisted largely of grasses--Bermuda, Dallis and carpet. The pasture contained numerous large trees which furnished shade utilized by the cows during the hot, sunshiny portions of the days.

RESULTS

I. *Daytime Changes in Body Temperatures and Respiration*

Indications of how cows react to changes in atmospheric temperatures during the daytime were secured from recorded changes in their body temperature and respiration and pulse rates during three days on pasture. In general, these changes showed increases (table 1) averaging for the day 1.8° F. in body temperature, 16 in respirations per minute, and only 1 in pulse rate per minute. The latter change was not significant.

TABLE 1

Reaction of milking cows to daytime changes in atmospheric temperatures

Period	Time of day	Air temp.	Relative humidity	Body temp.	Resp. rate	Pulse rate
		° F.	*%*	*° F.*	*per min.*	*per min.*
In milking barn	5: 45 A.M.	73.0	89.3	101.7	63	66
Cows enter shade	9: 20 A.M.*	80.0	81.3	102.4	64	67
1 hour in shade	10: 20 A.M.	83.1	74.3	102.6	71	66
In shade	2: 00 P.M.	86.7	73.0	103.3	78	68
In milking barn	3: 00 P.M.	86.0	65.3	103.5	79	67

* The time that cows entered shade varied from 8: 35 to 10: 15 A.M. with an average of 9: 20 A.M.

During the day atmospheric temperatures increased from an average of 73° F. when cows left the barn at 5:45 A.M. to 86.7° F. at 2:00 P.M., with the reading slightly lower, 86° F., at 3:00 P.M. when cows were put into the barn prior to the evening milking. During this same period the relative humidity had shown a progressive drop from 89.3 per cent to 65.3 per cent.

Between 5:45 A.M. and the time that cows entered shade (average time 9:20 A.M.) air temperatures increased from 73° to 80° F., while relative humidity dropped from 89.3 to 81.3 per cent. Body temperatures of the cows had increased during that time from 101.7° to 102.4° F., but the increase in respiration rate was negligible, from 63 to 64 per minute.

After one hour in the shade with an increase from 80° to 83.1° F. in air temperatures and a decrease from 81.3 to 74.3 per cent in humidity, the cows were apparently unable to eliminate heat fast enough to prevent further changes in their temperature and respiration. As a result, body temperatures increased to 102.6° F. and respiration to 71 per minute. At 2:00 P.M. this upward trend continued, for air temperatures had then reached 86.7° F. and the cows averaged 103.3° F. in body temperature and 78.2 in respiration rate. After cows entered milking barn at 3:00 P.M. the only significant further change was in body temperatures, which then averaged 103.5° F., the highest recorded for the day.

II. *Grazing Performance During 24-Hour Periods*

During five 24-hour days, September 10 to 14, 1945, the six cows were constantly observed while on pasture. For the purpose of this experiment the cows' day (24-hour period) was considered as beginning when they entered pasture following the morning milking. During these days atmospheric temperatures were recorded at intervals, *i.e.*, 8:30 A.M., 11:30 A.M., 2:30 P.M., 6:30 P.M., 12:30 A.M., and 3:30 A.M. These readings (table 2) show some striking variations within each 24-hour period as well as between periods.

In general, the temperatures for the first two days averaged relatively high during the daytime, 86° F. and 85° F., while for the third day they averaged slightly lower, 82° F. The last two days were much cooler, the temperature averaging 72° F. for each. The averages for night temperatures are much in the same order, with the exception of that for day number 2 which was lower than day number 3, due to the cooling effects of a shower at around 5:00 P.M.

The first two days of observation on grazing (table 3) gave results which appear to be representative of how milking cows perform on pasture during relatively warm weather. The daytime period consisted of that portion of the day between the time cows entered pasture in the morning (7:15 A.M.) and the time they left in the afternoon (2:35 P.M.). During this daytime period the cows grazed only 1.9 hours the first day and even less, 1.8 hours,

TABLE 2

Daily changes in atmospheric temperatures

Time of day	24-hour periods (days)					
	1	2	3	4	5	Average
	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*
Day						
8: 30 A.M.	80	79	75	70	65	74
11: 30 A.M.	87	88	83	72	75	81
2: 30 P.M.	90	88	87	75	77	83
Average	86	85	82	72	72	79
Night						
6: 30 P.M.	87	72	73	71	73	75
12: 30 A.M.	78	72	72	58	58	68
3: 30 A.M.	78	70	73	56	55	66
Average	81	71	73	62	62	70

the second day. As contrasted to this, time spent not grazing (mostly in shade) was 5.7 hours for the first day and 5.5 hours the second day. The daytime atmospheric temperatures averaged 86° and 85° F., respectively, for the two days.

The night totals for grazing of 6.5 and 6.2 hours for these first two nights revealed one of the unexpected findings from this study. Grazing at night for these warm days thus equaled more than three times that for the daytime.

For the third day the grazing record showed an intermediate status. Daytime grazing increased approximately 1 hour, to a total of 2.8 hours, while grazing at night fell off 1.1 hours.

The fourth and fifth days gave some striking evidence of how cooler weather affects grazing habits. Daytime grazing increased to 4.5 hours for each of these two days, or 2.4 times the average for the first two (warm) days. Night grazing decreased to 4.7 and 5.0 hours, respectively, for the last two days. In spite of this decrease the total grazing during 24 hours

TABLE 3

Grazing and air-temperature relationships (average of 6 cows for daytime and night)

24-hour periods	Average air temperatures*		Hours in pasture	Hours spent grazing or not grazing					
				Daytime		Night		Daily totals	
	Day-time	Night		Grazing	Not grazing	Grazing	Not grazing	Grazing	Not grazing
days	°*F.*	°*F.*							
1	86	81	17.2	1.9	5.7	6.5	3.1	8.4	8.8
2	85	71	17.3	1.8	5.5	6.2	3.8	8.0	9.3
3	82	73	17.2	2.8	4.4	5.1	4.9	7.9	9.3
4	72	62	16.9	4.5	2.7	4.7	5.0	9.2	7.7
5	72	62	16.8	4.5	2.8	5.0	4.5	9.5	7.3

* Air temperature readings are averages of those taken at 8: 30, 11: 30, and 2: 30 during the daytime and 6: 30, 12: 30, and 3: 30 during the night.

for these cooler days showed 9.2 hours and 9.5 hours, or more than 1 hour longer than for the first three days.

The number of grazing periods each cow had for day and night is shown for each of the five days in table 4. While variations existed, in general, it can be seen that one grazing period during the day was most common, and the average was 1.4. At night three periods were most often spent grazing and the average was 2.7.

TABLE 4

Number grazing periods during day and night (summary of five 24-hour periods for 6 cows)

24-hour periods	Daytime							Night							Daily average
	Cow number						Average	Cow number						Average	
	1	2	3	4	5	6		1	2	3	4	5	6		
days															
1	2	2	1	3	2	2	2.0	3	2	3	2	3	3	2.7	4.7
2	1	1	1	1	1	1	1.0	3	3	2	3	3	3	2.8	3.8
3	1	2	1	1	1	1	1.2	3	4	2	2	3	3	2.8	4.0
4	2	1	2	1	2	2	1.7	3	3	3	3	2	2	2.7	4.4
5	2	1	1	1	2	1	1.3	2	2	2	3	2	1	2.3	3.6
Average	1.6	1.4	1.2	1.4	1.6	1.4	1.4	2.8	2.8	2.4	2.6	2.6	2.4	2.7	4.1

In each case the cows had their longest grazing periods immediately after being turned into pasture following milking. In the daytime the early morning grazing was the only period that cows grazed a sufficiently long time to make a major contribution. Whenever a cow grazed a second or third time during the daytime, it was for only a short time and usually during either a cloudy period or in some spot shaded by trees.

Night grazing started off in all cases by the long first period, averaging 3.1 hours. Following this the cows spent an average of 1.2 hours lying down, standing, chewing cud, etc., prior to the second grazing period. With the exception of one cow during one night, they then all grazed for a second period, averaging 1.4 hours. When the first two grazing periods were long, the cows sometimes omitted further grazing. However, the records showed that for an average of three of the five nights the six cows grazed during a third period averaging 1.5 hours. One cow during one night had a fourth grazing period of 3 minutes. While much variation occurred between cows and for different nights, the first grazing periods began at 5:45 P.M. on the average; the second around 10:30 P.M.; and the third, when it occurred, started at approximately midnight. Cows were taken to the milking barn at 3:30 A.M.; otherwise, there may have been more third and fourth grazing periods.

On an average, the cows spent 4.2 hours not grazing during the night. Variations among the six cows (table 5) ranged from 3.5 to 4.9 hours. Time

TABLE 5

Nongrazing record during night (average of 5 nights)

Cow No.	Lying down	Standing	Total
	hours	*hours*	*hours*
1	2.8	1.5	4.3
2	3.0	0.8	3.8
3	4.4	0.3	4.7
4	3.1	0.4	3.5
5	4.1	0.8	4.9
Average	3.5	0.7	4.2

spent lying down ranged (between cows) from 2.8 to 4.4 hours and averaged 3.5 hours. Standing time not grazing ranged from 0.3 hours to 1.5 hours, with an average of 0.7 hours.

DISCUSSION

Results secured from this study should have general application to much of the United States, inasmuch as daytime atmospheric temperatures during the course of the experiments were not extremely high or extremely low, varying from 65° to 90° F. These findings, which show to what extent cows became uncomfortable while grazing in the sunshine and how that tended to shorten their grazing period, suggest a need for improvements in summer management of dairy cattle.

Cows observed in this study entered shade on relatively warm days when their body temperatures averaged 102.4° F. This was an increase of 0.7° F. over their near-normal temperature (2) of 101.7° F. when they were turned to pasture at 5:45 A.M. Respirations per minute during this same period had increased from 63 to 71. Pulse-rate observations showed practically no change during this period. It seems obvious that shade tended to make the cows more comfortable even though further increases in body temperature and respiration rates were observed as the day progressed up until 3:00 P.M. During this same period atmospheric temperatures also increased.

Daytime grazing during the two warm days under observation was surprisingly short (1.9 and 1.8 hours). Cows took shelter under trees at around 9:00 A.M. and refused in most cases to venture forth into the sunshine in order to graze, but instead waited until night when they grazed for periods exceeding the daytime periods by a ratio of more than 3 to 1. Two cool days, averaging 72° F., which was 14° and 13° F. lower, respectively, than the two warmer days, caused a significant change in the grazing schedule. Daytime grazing increased around 2.5 times and night grazing decreased some, yet the 24-hour totals showed increases in grazing time due to cool weather of more than 1 hour daily.

The increased activity of cows on nights following warm days indicates the importance of allowing cows access to good night pasture. The plan of leaving cows overnight in a dry lot or on poor pasture so that they will be convenient to the barn in the morning is most certainly a poor management practice. The daytime grazing data for warm days show that cows should be turned into the very best pasture during the forenoon if they are to become filled before the heat of the day forces them to seek shade. In fact, if it is a choice between a fair and an excellent pasture, it would seem best to use the excellent pasture during the day and use the fair pasture at night when cows will spend more time grazing to get their fill. The short daytime grazing period indicates a need for experimental work to test how best to furnish cows supplemental feed during their long period in the shade. Likewise, tests are needed on what practical methods can be used to maintain lower body temperatures and respiration rates during the times cows are in the shade.

SUMMARY

Observations on 3 Jersey and 3 Holstein milking cows during the summer of 1945 at Baton Rouge, Louisiana, gave the following results:

1. Cows showed a progressive rise in body temperature throughout the day. When they left the milking barn at 5:45 A.M. it averaged 101.7° F.; upon entering the shade of trees at 9:20 A.M. it was 102.4° F.; after a period of one hour in the shade, 102.6° F.; at 2:00 P.M., 103.3° F.; and upon entering milking barn at 3:00 P.M it was highest, 103.5° F. During these same respective periods respiration rates were 63, 64, 71, 78, and 79 per minute Atmospheric temperatures during these periods increased from 73.0° F. at 5:45 A.M. to a high of 86.7° F. at 2:00 P.M.

2 Observations of 24-hour grazing periods showed that during two relatively warm days cows grazed less than two hours, 1.9 and 1.8 hours during daytime (between A.M. and P.M. milking periods) but grazed three times as much, 5.7 and 5.5 hours, at night (between P.M. and A.M. milking). Data on two cool days showed daytime grazing 2.4 times as great as for warm days, and 24-hour grazing totals more than one hour longer than for warm days.

3. The number of grazing periods averaged 1.4 for daytime and 2.7 for night. Cows seldom had but one important grazing period during the daytime. Three grazing periods were the most common at night and the time spent grazing averaged 5.5 hours, while 3.5 hours were spent lying down and 0.7 hours standing without grazing.

4. Results of the study suggest the need for good pasture at night and, especially on warm days, an excellent daytime pasture. There appears to be a need for experimental trials to test the best method of providing supplemental feed to cows during their long periods in shade during warm summer days, and to determine the best methods for making them more comfortable during this same period.

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THE OXYGEN CONTENT OF THE ATMOSPHERE IN CONTAINERS OF DRIED MILK PACKED IN NITROGEN

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Previously reported experimental data indicate that the rate of autoxidation of milk fat varies directly with the oxygen concentration (4) and that the keeping quality of dried milks is increased greatly by reducing the oxygen concentration of the container atmosphere to 3 per cent or less (2, 4). A concentration of 3 per cent of oxygen in a container of dried milk is equivalent to 0.0324 ml. per gram of powder, or 0.125 ml. per gram of fat, or 11.5 per cent of the volume of the fat.

Lea, Moran, and Smith (3) studied the keeping quality of dried milks packed in atmospheres of 0.01, 0.02, 0.03, and 0.04 ml. of oxygen per gram of product or approximately 1.0, 2.0, 3.0, and 4.0 per cent of oxygen in the atmosphere of the container and concluded that oxygen concentrations below 2 per cent in the containers can be considered good and those below 1 per cent ideal for commercial packing. These concentrations of oxygen in the atmosphere of the container of dried milk are approximately 7.66 and 3.83 per cent, respectively, of the volume of the fat, which is greatly in excess of that necessary to render the fat tallowy (approx. 0.80 per cent by volume) if all of the oxygen reacted with the fat (4). However, as pointed out by Lea, Moran, and Smith (3), and also as we observed in our studies, the skim milk solids absorb oxygen and hence the actual percentage of oxygen in the container which will cause spoilage through autoxidation of the fat will be greater than the value indicated unless the fat is of such inferior quality that absorption of oxygen by it begins immediately or shortly after packing of the dried milk. The actual percentage of oxygen in the container which will cause spoilage through autoxidation should be expected to vary somewhat, therefore, with the quality of the milk and the processing treatment used in the manufacture of the product. In a sample used in our studies on the relationship of percentage of oxygen to keeping quality, the value seemed to be approximately 1.33 per cent (4).

Theoretically, evacuation to approximately 109, 72, and 36 mm. pressure should produce concentrations of oxygen of 3, 2, and 1 per cent respectively in the gases of the container. Evacuation to 10 and 5 mm. pressure should produce oxygen concentrations of approximately 0.28 and 0.14 per cent respectively.

$$\text{Per cent oxygen (volume)} = \frac{\text{Pressure used (mm.)}}{760} \times 20.9$$

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Thus, so far as the oxygen in the atmosphere of the container is concerned, it can be reduced readily to a low value. However, dried milks which have been subjected to evacuation contain sorbed gases which desorb slowly and diffuse into the inert gas and thus increase the oxygen content of the container atmosphere.

The rate of desorption has been studied by Lea, Moran, and Smith (3) and by Coulter and Jenness (1). Results obtained by them indicate that an equilibrium was reached in practically 5 to 7 days, though in some cases the oxygen concentration increased slightly with storage beyond these time periods.

With the use of two cycles of evacuation for 5 min. at 2 mm. pressure and an intervening storage period of at least 5 days, Lea, Moran and Smith (3) found that final oxygen concentrations of less than 2.0 per cent could be obtained. Similar results were obtained by Coulter and Jenness (1) with the use of a pressure of 1 and of 10 mm. and a 7-day intervening period of holding. The latter authors found also that the degree of percentage oxygen reduction varied somewhat with the two commercial samples used.

Experiments of this nature and similar in many respects to those of Lea, Moran, and Smith (3) and of Coulter and Jenness (1) have been carried on in these laboratories for the past two years, the specific aim being to determine the percentage oxygen concentration attainable in the atmosphere of containers of dried milk under varied conditions of pressure, temperature, holding time, and with different periods of storage.

EXPERIMENTAL

Single stage packing. Cans of 160 ml. capacity were packed with 86 grams of dried milk, sealed, tested for leaks, a hole of ¼ inch diameter was punched in the cover of each can, and the cans were placed in a large desiccator which served as an evacuation chamber.

The desiccator was then evacuated at different degrees of pressure for various lengths of time and at different temperatures, and subsequently filled with nitrogen. The cans were removed from the desiccator, solder-sealed and stored at room temperatures. At regular intervals the percentage oxygen concentration of the container gases was determined. The results upon a representative sample of commercial dried milk are shown in figure 1.

The results emphasize the importance of evacuation at relatively low pressures and agree with the conclusions from former experiments that increases in the time of evacuation from 3 to 30 minutes and increase in the temperature of evacuation decrease relatively slightly the sorbed gases in dried milk.

This is indicated more specifically by the values in table 1 obtained after 8 days of storage of the packed samples.

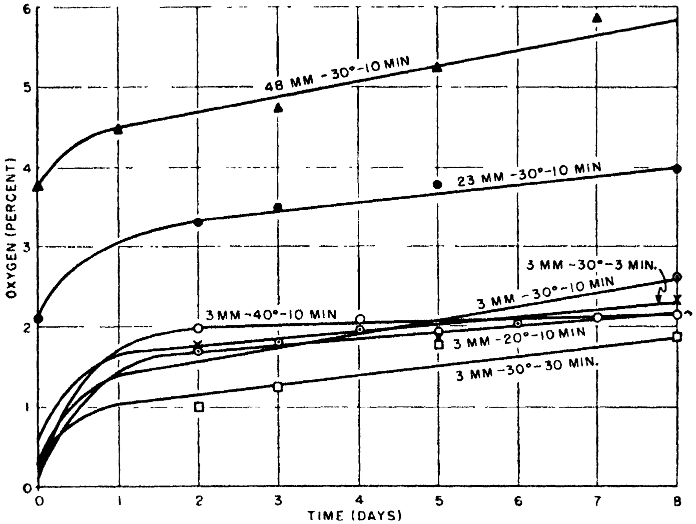

FIG. 1. The per cent oxygen in the atmosphere of containers of whole milk powder which had been evacuated under varied conditions of time, pressure and temperature, and stored for different periods of time.

Multiple-stage packing. The efficiency of oxygen removal by successive evacuations with intervening periods of 3 and 4 days to allow for desorption and diffusion is indicated in figures 2 and 3.

The relative efficiency of successive stages of evacuation and desorption is shown in figure 4, wherein are plotted the percentage oxygen concentration values after 3 and 4 days following each evacuation, as given in figure 3. The relatively high efficiency of a second evacuation over subsequent evacuations in lowering the final oxygen concentration of the containers is clearly indicated.

As indicated in figure 3, after 4 evacuations the final percentage oxygen in the container gas increased but slightly over that attained after the third

TABLE 1

The per cent oxygen in the atmosphere of containers of whole milk powder which had been evacuated under varied conditions of time, pressure and temperature

Pressure	Time	Temperature	Per cent oxygen in the head space	
			Before storage	After 8 days
mm.	*Min.*	°*C.*		
48	10	30	3.8	5.8
23	10	30	2.1	4.0
3	3	30	0.5	2.3
3	10	30	0.3	2.6
3	30	30	0.45	1.8
3	10	20	0.14	2.1
3	10	40	0.08	2.1

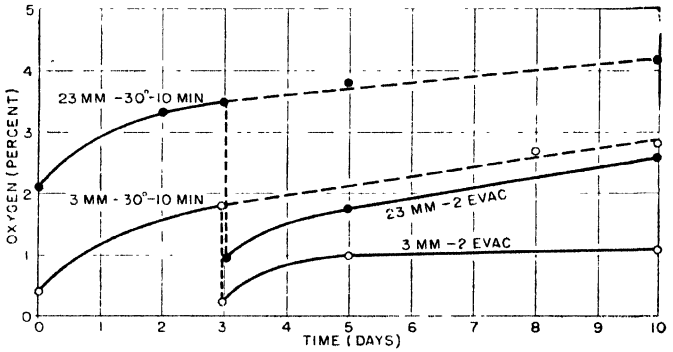

FIG. 2. The per cent oxygen in the atmosphere of containers of whole milk powder in which a second evacuation followed the first after a 3-day storage period.

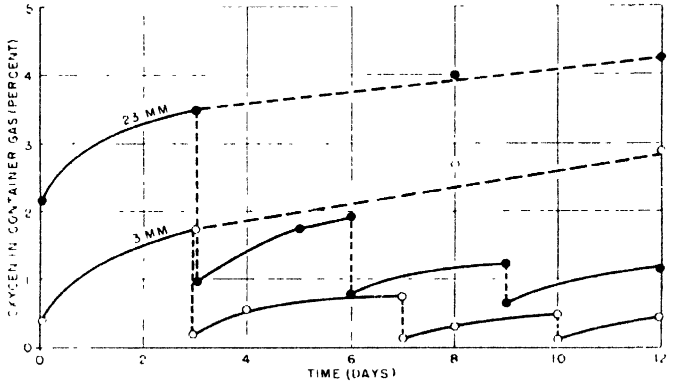

FIG. 3. The per cent oxygen in the atmosphere of containers of whole milk powder after each of 4 evacuations, with intervening storage periods of 3 and 4 days.

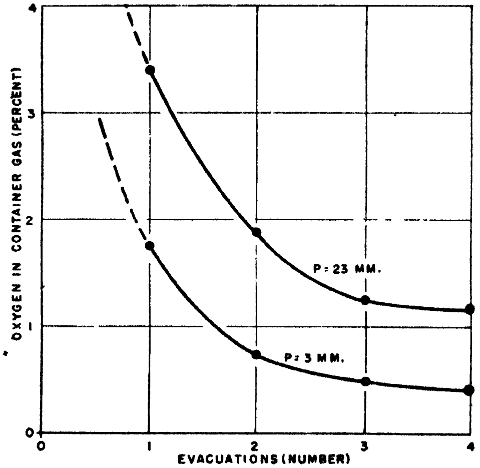

FIG. 4. The per cent oxygen in the atmosphere of dried whole milk containers after 3 or 4 days storage following each successive evacuation.

stage in 3 days of holding. Since, according to the data in figures 1 and 2, equilibrium of the gases is not reached in 3 or 4 days and since some of the sorbed gases are removed during each evacuation, the values given are not absolute but are those which can be attained under the conditions stated. The percentage oxygen in the container would no doubt increase somewhat more if the holding periods were longer, since, as indicated in the results obtained in these laboratories, some oxygen can be removed from dried milk with a Toepler pump at 70° C. after the rate of desorption becomes practically zero. If equilibrium had been attained after each evacuation, the percentage oxygen removed with each evacuation would have been constant and the rate of decrease of the final oxygen content in the containers would have been logarithmic. Under the experimental conditions used, this rate of decrease was not obtained.

SUMMARY

The results confirm those previously obtained by direct measurement of the amounts and composition of the container gases, with respect to the relative importance of time, temperature, and degree of evacuation in reducing the oxygen concentration of the container gases. That the amounts of adsorbed gases do not decrease to a great extent with increases in the value of these factors within practical limits is also indicated by the desorption values obtained. If equilibrium had been attained in each case, the rate of decrease of the final oxygen content in the containers should have been of a logarithmic nature, since the percentage oxygen removed in each evacuation is a constant. However, the rate obtained practically is not of a logarithmic nature, due presumably to the fact that the values for the per cent oxygen in the container atmospheres are not equilibrium values.

The relatively high efficiency of a second evacuation stage in decreasing the per cent oxygen content of the container gases is indicated. However, the rate of desorption seems to vary with different dried milks; hence, the efficiency of gasing procedures should be established for the product concerned in each case.

In our experience the use of two stages of evacuation at 3 mm. pressure with a 3- to 4-day intervening holding period will produce a final oxygen concentration in the container of approximately 1 per cent. Increasing the duration of the holding period will reduce further the final oxygen concentration.

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FACTORS WHICH CONTRIBUTE TO THE PHYSICAL STABILITY OF FROZEN CREAM

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Differences in milk, especially in the size and number of the fat globules, and changes in the temperature of the milk and cream, greatly influence the stability of the cream emulsion after freezing and brief storage as shown in an earlier publication (2).

Nine grams of cream frozen in a Babcock cream test bottle was the experimental unit, the freezing time was 30 to 40 minutes, the temperature of storage was − 15° C. and the duration of storage was less than 48 hours.

In the work which now will be described, about 150 ml. or one-third of a pint of cream in a small can was the experimental unit and the storage period was from less than two days to several months. Data were obtained on the influence of various factors, not only upon the stability of the emulsion, but also upon the body of the cream.

Canned cream was frozen in air at − 17° C., and in ethyl alcohol at − 17° C. and − 29° C. Solid carbon dioxide was used to maintain the alcohol baths at − 17° C. and − 29° C. and to agitate them during freezing of the cream. Under these conditions a sample became solid in 4 hours, 50 minutes, and 25 minutes, respectively. Cans were stored in air at − 9° C. and − 17° C. and in alcohol at − 29° C.

The emulsion stability of each sample was obtained by weighing 9 grams of the frozen cream into a funnel placed in a cream test bottle, washing the cream into the bottle with lukewarm water and proceeding as described by Bell and Sanders (2). Baldwin (1) according to Trelogan and Combs (5) secured results indicating that cream containing more than 30 per cent butterfat tended to freeze homogeneously and such cream could be accurately analyzed for fat by taking a sample from any part of the unfrozen or frozen portion.

Viscosity measurements were made with a MacMichael viscosimeter. Each sample was prepared for this measurement by leaving the opened can at room temperature for 2 hours, stirring the cold cream, placing the can in water at about 32° C., and warming the cream with occasional moderate agitation to 30° C. in the course of 30 to 45 minutes.

Forty per cent cream was used in most of the experiments. All cream was pasteurized by heating to 80° C. and cooling promptly to not lower than 30° C.

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EXPERIMENTAL RESULTS

Quick-freezing versus slow-freezing and variations in storage temperature with cream from cooled herd milk. The milk was a mixture of that obtained from Holstein, Jersey, and a few other cows of the Bureau of Dairy Industry herd at Beltsville, Maryland. It contained 4.2 per cent fat and was considered fairly representative in composition of herd milk that is delivered to factories in this country.

This morning milk was cooled to 10° C. and delivered to the Bureau's research laboratories in Washington, D. C., where it was warmed to 38° C., separated and the cream standardized to 40 per cent fat content with the warm skim milk. The raw cream was pasteurized, cooled to 30° C. and placed in the small cans. The freezing conditions were (1) overnight in air at – 17° C., (2) 2 hours in alcohol at – 17° C. and (3) 1 hour in alcohol at – 29° C. The storage temperatures for the samples frozen under each of these 3 conditions were – 9° C., – 17° C. and – 29° C.

Representative samples were removed from storage and inspected on the second day after the cream was frozen.

Only those samples which had been frozen quickly at – 17° and – 29° C. and stored at the latter temperature were unchanged in appearance after thawing. At this time and at later dates covering a period of over 3 months, samples prepared and stored under these conditions thawed into fluid homogeneous creams. Their initial emulsion stabilities, expressed as the percentage of the total fat which oiled off after centrifuging, were 45 and 20. During the next 3 months these figures increased to 60 and 40 per cent, respectively.

The initial viscosity values at 30° C. of these 2 satisfactory samples were 24 and 20 centipoises.

The other 7 samples thawed into semisolids which, after stirring, were flaky or coarse in texture and oiled off from 70 to 100 per cent. Samples of these creams which were examined during the next 3 months had similar body properties.

Cooled milk of relatively high fat content is not, in our experience, a good source of cream that is to be preserved by freezing and cold storage. Therefore, the milks in the following experiments were obtained from Holstein cows. If the differences for low fat milk which are shown in the figures had been obtained and plotted for creams from high fat milk, they would have been of greater magnitude, not as easy to present and less reliable because of the inferior body of some of the samples.

Cooled versus warmed low-fat milk. This morning milk was obtained from Holstein cows which were known to give milk of low fat content. It contained 3.1 per cent. Half of it was cooled to 10° C. and half was warmed to 43° C. before it was sent to the laboratory. Each half was separated at 38° C. and the cream standardized to 40 per cent with the skim milk. After pasteurization in the usual manner, cans of each warm cream were frozen in alcohol at – 17° C. and at – 29° C. with the aid of solid carbon dioxide and stored at these temperatures.

Figure 1 shows the viscosity and emulsion stability values of these thawed samples over a period of 22 weeks.

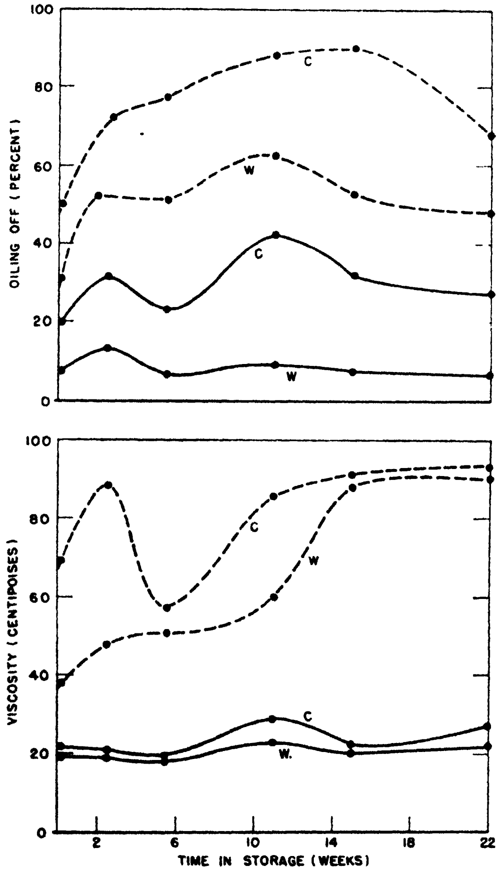

FIG. 1. Viscosity and oiling off values of 40 per cent cream from 3.1 per cent milk, part of which was cooled to 10° C. and part warmed to 43° C. "C" refers to cooled milk cream and "W" to warmed milk cream. Solid lines represent creams quick-frozen and stored at – 29° C.; broken lines refer to creams quick-frozen and stored at – 17° C.

The samples from cooled milk that were frozen in less than an hour in alcohol at – 17° C. and held at – 17° C. thawed into coarse and milky creams; those from warmed milk were more homogeneous but not of good body. In contrast the samples frozen quickly at – 29° C. and stored at – 29° C. thawed into fluid and homogenous creams. Samples derived from the cooled milk thawed at a slower rate and into slightly more viscous samples than did those from the warmed milk. Oiling off values are more reliable than are those for viscosity, especially in instances in which the cream did not become homogeneous on thawing and stirring.

Uncooled versus cooled cream. This 40 per cent cream was a mixture of that obtained from warmed morning Holstein milk of 3.4 per cent fat content and from Holstein milk separated the day before and cooled to 10° C. for preservation. The latter was warmed to 30° C. before it was mixed with the fresh cream.

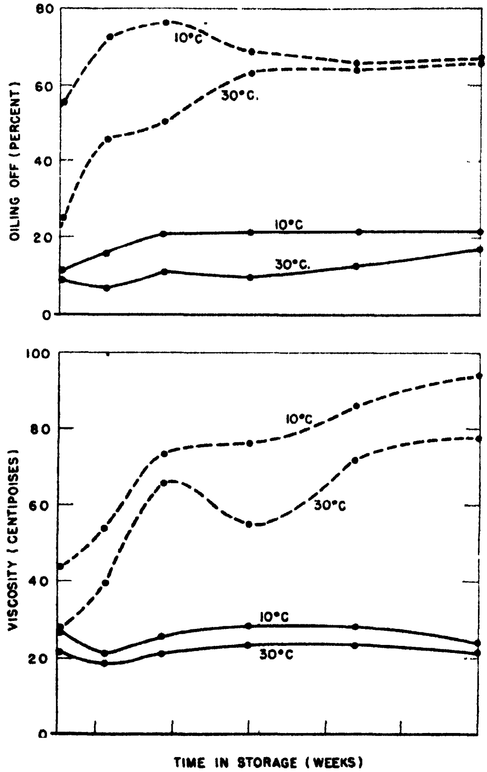

FIG. 2. Viscosity and oiling off values of 40 per cent cream, part of which was canned while still warm (30° C.) and part tempered to 10° C. over a surface cooler prior to canning. Solid lines represent samples quick-frozen and stored at – 29° C., broken lines refer to creams quick-frozen and stored at – 17° C.

Part of the cream was canned while still warm; the remainder was chilled over a tubular surface cooler to 10° C. and then canned. Samples were frozen in alcohol at – 17° and at – 29° C. Those samples which were frozen at – 17° C. were stored at that temperature; those at – 29° C. were stored at – 29° C.

Viscosity and oiling off values are shown in figure 2.

On the initial inspection, the 2 samples that had been stored at – 17° C. were coarser and more viscous than the 2 that had been stored at – 29° C.

They had a body resembling that of cultured butter-milk. The same was true but to a lesser degree of the 2 samples that were chilled over a surface cooler relative to the 2 that were not cooled before they were canned.

The samples frozen and stored at – 29° C. were superior in body and emulsion stability to those frozen at – 17° C. and stored at that temperature. As between the 2 frozen and stored at – 29°, the one which had been tempered to 30° thawed sooner and more completely into a fluid than did the one that was cooled to 10° prior to canning and freezing. However, each was a homogeneous, satisfactory cream.

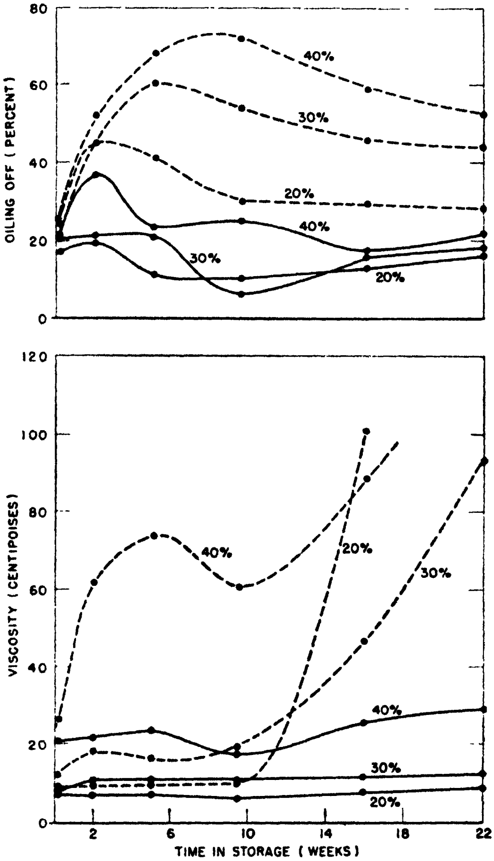

FIG. 3. Viscosity and oiling off values of creams of 40, 30 and 20 per cent fat content prepared from warmed Holstein milk. The cream was not cooled but was packaged while warm. It was quick-frozen and stored at – 17° C. (broken lines) and at – 29° C. (solid lines).

Viscosity values for the samples frozen at – 17° C. are not as reliable as those for the other samples because of partial separation of a discontinuous phase.

Creams of 40, 30 and 20 per cent fat content. Holstein milk containing 3.7 per cent fat was warmed to 43° C. and delivered to the Research Laboratories within 3 hours of milking. Its 40 per cent raw cream was divided into 3 parts. One was diluted with the fresh skim milk to 30 per cent fat content, another to 20 per cent and the 3 creams pasteurized and cooled to 30° C.

The samples were frozen quickly. Half of them were stored at – 17° C. and half at – 29° C.

Viscosity and oiling off values are presented in figure 3.

Samples stored at – 17° C. thawed at room temperature within 2 to 3 hours into fluid, homogeneous creams. Those stored at – 29° C. thawed into fluids somewhat sooner. The superiority of the latter samples was evident after the various samples had been in a 10° C. room overnight. They remained homogeneous whereas there was an upper layer and a milky lower portion in the others.

Before thawing, the 20 per cent and 30 per cent creams were not as attractive as those which contained 40 per cent fat. They were coarse and more icy and, of course, on thawing they were less viscous or creamy.

The data show that the creams of 20, 30 and 40 per cent fat content were preserved equally well at – 29° C. when advantage was taken of factors which contributed to the preservation of the physical stability of the freshly drawn milk and the cream.

DISCUSSION

Oiling off following freezing of cream is the result of coalescence of fat globules. According to Sommer (4):

> Freezing tends to break the fat emulsion especially in a product rich in fat such as the high fat cream used for frozen storage. The destabilization of the protein adsorption film may be involved, but the main factor appears to be the physical crowding of the fat globules as water is converted into ice. If the ice crystals are large in relation to the size of the fat globules, then there will be a large number of globules crowded together between adjacent ice crystals, the globules are inevitably deformed, fat to fat contact is likely to occur, and on later melting the fat naturally flows together to form a single large oil droplet.

It follows, therefore, that if cream is to be preserved unchanged in a frozen state it should be prepared and stored under conditions which will minimize the tendency of the fat globules to coalesce. Ideal freezing conditions would convert cream into a glass so quickly that all particles would be fixed in position. In thawing, the reverse would be ideal and after thawing orientation of all particles would be the same as it was before the cream was frozen. On the one hand heat would leave the liquid quickly and uniformly and the liquid would change into a homogeneous solid, and on the other, heat would return at a rapid and constant rate throughout the mass and reconvert the mass into a homogeneous liquid.

Thus the importance of the degree of dispersion of fat globules just prior to freezing cream becomes obvious. If the fat is already clumped, advantages of quick freezing will be only partially realized.

It has been shown (2) that 40 per cent cream from Holstein milk oils off after freezing less than does 40 per cent cream from Jersey milk, that the cream should be derived from milk that has not been cooled and the fresh cream should be warm at the start of the freezing operation. Such cream is fluid rather than viscous. Its fat globules have not been permitted to become semisolid and to clump. This is the reverse of the way to make cream appear rich and of heavy body. Viscous, thick cream is made by starting with milk in which the fat globules are large, and by causing the globules to form aggregates through cooling and holding of the milk and of the cream (3). This is not to say that high viscosity in cream is due to fat clumping only. Other concurrent changes contribute to the increased internal friction.

Whenever a sample thawed into a semisolid or viscous cream which did not have a good body, a large proportion of its fat oiled off. Whenever a sample thawed into a fluid, it had a low viscosity and oiled off only a little.

The data indicate that quickly frozen cream which does not oil off, is homogeneous, and has a low viscosity after brief storage, will retain its physical properties throughout a long storage period if a sufficiently low temperature is maintained.

Neither this paper nor the preceding one describes how to increase the resistance of a freshly separated cream to the disruptive forces of freezing and thawing. They do indicate, however, the importance of certain properties of the fresh milk and how to preserve them for the purpose of making a stable frozen cream.

CONCLUSIONS

Factors which contribute to the physical stability of frozen cream are:

(1) The use of freshly drawn milk which contains small, unclumped fat golbules.

(2) Maintenance of these globules in a fluid, completely dispersed state until freezing time.

(3) Quick freezing to retain this degree of dispersion.

(4) A storage temperature sufficiently low to prevent the physical changes which cause localized crowding, distortion and disruption of the fat globules and consequent oiling off on thawing.

Frozen cream which is prepared and maintained under these conditions will have a stable emulsion and a good body when thawed after several months storage.

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THE EFFECT OF MILK PRODUCTS ON THE HEAT-STABILITY AND VISCOSITY OF CREAM-STYLE FOODS

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Milk and cream are sometimes used in canned foods to improve flavor, body and nutritive value. Typical of these heat-processed foods are the cream soups. Other products such as canned white sauce and creamed vegetables have not been produced commercially on a large scale.

This investigation is concerned with the effect of variations in the composition and processing of simple cream-style mixtures upon their heat stabilities and viscosities. A better understanding of the behavior of milk constituents in the cream-style foods may assist in improving them and in extending the use of milk products to new canned foods. The ingredients of the sterilized milk foods which affect their heat stability and viscosity are the fat and protein of the milk, the starch of flour added as a thickener and binder, the salt and the type of vegetable or flavoring material which is used.

EXPERIMENTAL

Milk solids were largely derived from pure butter oil and specially dried samples of skim milk. These products provided a source of supply of milk constituents which did not change during the work. The conditions of manufacture and the heat stability of the dried skim milks are given in table 1.

Each starch-milk-salt mixture was heated with stirring to 176° F. (80° C.), canned and sterilized at 239° F. (115° C.) for the time specified, unless otherwise indicated. The canned samples were cooled, held overnight at 86° F. (30° C.), and observations on body and viscosity made the next morning.

Heat-stability data were obtained by heating the fluid products in small tins (208 × 208) in a pilot evaporated-milk sterilizer with the reel revolving at 4 r.p.m. The temperature of heating was 239° F. (115° C.) and the results were expressed as the time in minutes to develop a visible coagulation. The experimental error for this determination was ± 3 per cent of the values reported. Samples for viscosity determinations were sterilized in the same way. The sterilizer reel was kept in motion during processing unless otherwise indicated.

Viscosity was measured at 86° F. (30° C.) by means of a McMichael viscosimeter with standardized wires. Measurements were made in a uniform manner and without excessive agitation. The results were expressed

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TABLE 1

The effect of the pre-drying heat-treatment of skim milk on the heat-stability of its dried and reconstituted product

Sample No.	Pre-drying treatment					Heat-stability at 239° F. (115° C.) of powder reconstituted to 9% solids
	Forewarming before condensing		Condensed to T.S.	Forewarming concentrate before drying		
	Temp.	Time		Temp.	Time	
	°F.	min.		°F.	min.	min.
Spray 1	149	20	36	122	0	134
Spray 2	203	10	36	122	0	89
Spray 3	182	30	36	182	10	127
Spray 4	149	20	27	122	0	387
Spray 5	149	20	27	203	10	30
Roller 6	149	20	27	122	0	0
Roller 7	150	30	27	122	0	0

Samples 1, 2, 3, and 6 were from one batch of skim milk, samples 4 and 5 were from a second and sample 7 from a third lot of milk. Powders 1 to 5 were spray dried under normal operating conditions on a 9-foot Gray-Jensen unit. Samples 6 and 7 were drum dried on a laboratory size atmospheric double roll drier.

in centipoises but the values were not absolute. Plastic effects which were not measured were observed in most of the samples. The viscosity figures were a good indication of the "body" or "apparent" viscosity of the mixtures.

The quinhydrone electrode was used in the measurement of hydrogen-ion concentration.

RESULTS

The effect of starch and salt upon the heat-stability of the milk in a simple fat-free mix is shown in table 2.

Short patent and standard patent flours obtained from the states of Minnesota, Kansas, Texas and Washington, were tested in mixtures of 2 per cent flour and 8 per cent skim milk solids. The heat stability of the 8 per cent solids control milk at 239° F. (115° C.) was 235 minutes, whereas the samples containing flour coagulated in 54 to 77 minutes. Differences in the lowering of heat stability by the eight samples of flour thus tested were not very important.

TABLE 2

Effect of corn starch (#1) and salt upon the heat-stability of a reconstituted dried skim milk (milk #1)

Sample No.	Composition of mixture	Heat-stability at 239° F. (115° C.)
1	Milk solids concentration—9%	134
2	“ “ “ —4%	285
3	4% M.S.N.F. + 2% corn starch	241
4	“ “ + ½% NaCl	129
5	“ “ + { 2% corn starch ½% NaCl	24

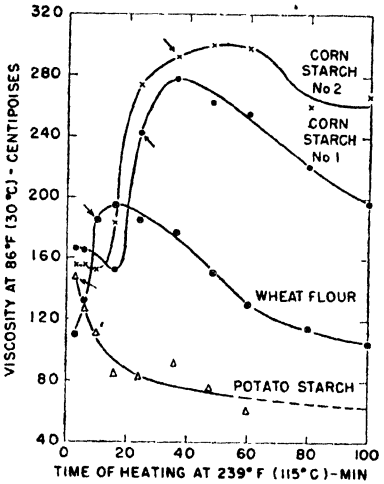

FIG. 1. The effect of different thickening agents upon the viscosity and heat-stability of some fat-free mixtures. Arrows indicate first visible casein coagulation. Each mixture contains 2% starch or $2\frac{1}{2}$% flour, $\frac{1}{2}$% salt and 4% solids derived from dry skim milk #1 (table 1).

A comparison of the effect of different starches and a sample of hard wheat, short patent flour upon the heat-stability and viscosity of some simple skim milk mixtures is presented in fig. 1.

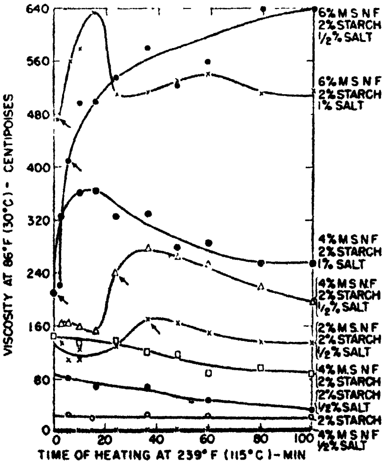

FIG. 2. The effect of increasing quantities of milk-solids-not-fat (M.S.N.F.) upon the body-forming properties of creamed bases. Arrows indicate first visible casein coagulation. No arrow indicates absence of coagulation. Skim milk #1 (table 1) and starch #1 (fig. 1) were used.

Figures 2 to 6 show the differences in kind and in quantity of the various components upon the heat-stability and viscosity of some basic mixtures. The data of figures 4, 5, and 6 were from mixes which contained fat and these were homogenized. The effect of different homogenization and sterilization procedures is shown in figures 5 and 6.

Data on the effect of different vegetable extracts upon the heat-stability of skim milk are given in table 3. Clear extracts were prepared by cooking the vegetable with an equal weight of water and filtering the mixture.

TABLE 3

The effect of the addition of different vegetable extracts upon the heat-stability of skim milk

Vegetable extract*	Reaction		Heat-stability at 239° F. (115° C.)†
	Aqueous extract	Milk-juice mixture†	
	pH	*pH*	*minutes*
No extract (equal parts of milk and water)		6.63	269
Stabilizing extracts:			
Cauliflower	6.04	6.59	448
Corn	7.38	6.73	518
Mushroom	6.20	6.49	> 550
Potato	6.08	6.62	435
Spinach	6.21	6.90	> 550
Destabilizing extracts:			
Cabbage	5.55	6.48	193
Celery	5.50	6.48	131
Onion	5.08	6.40	25
Pea	6.25	6.48	189
Tomato	4.48	6.18	0
Extracts with little effect:			
Asparagus	6.00	6.60	290
Beet	5.43	6.52	317
Carrot	5.18	6.49	279
Turnip	5.37	6.40	276

* The extract was obtained by cooking together equal weights of vegetable and water and filtering the mixture.

† This was a milk-juice mixture which contained equal weights of fresh skim milk and vegetable extract.

Tomato was found to destabilize the milk more than any other vegetable, because of its high acid content. Filtered tomato serum at pH 4.25 lowered the heat-stability of dilute skim milk with which it was mixed unless the serum was neutralized to a reaction above pH 5.7 to 5.9. The extent of neutralization of the tomato serum which was necessary to prevent it from affecting heat stability was dependent upon the concentrations of serum and skim milk and of the natural characteristics of the milk.

The presence of an inert phase produced a slight lowering in heat-stability as indicated by the data of table 4. Inert material such as tomato pulp or ground filter paper caused a fractional coagulation of the milk casein during sterilization of the mixtures. The progressive increase in the coagulum with increases in heating time made difficult any precise determination of heat stability.

The manufacture of cream-style soups was studied. Satisfactory formulas included 3 per cent to 4 per cent milk-solids-not-fat, 2 per cent to 4 per cent milk fat, 2 per cent to 3 per cent corn starch or flour, about 1 per cent salt and the remainder vegetable extract, vegetable pulp and soup stock. Homogenization of the basic mix, previously freed of large pieces of pulp or other material which could not be homogenized, was conducive to the production of a smooth body. The mix containing the starch was heated to a temperature not above 113° F. (45° C.) before homogenization. The canned soup was heated to at least 240° F. for 60 minutes, the exact conditions depending upon the material in the can.

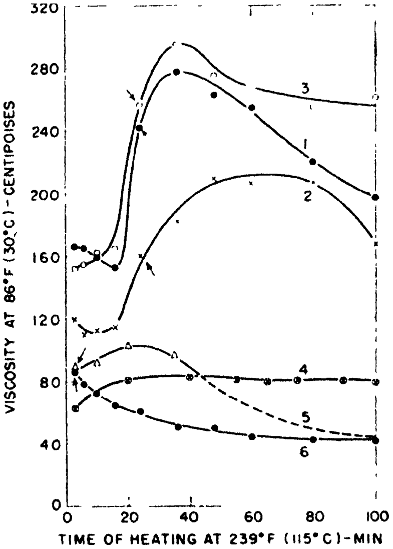

FIG. 3. The effect of the heat-treatment of skim milk before concentrating and before drying upon its heat-stability and viscosity-forming properties in creamed bases. Arrows indicate first visible casein coagulation. Milk #4 did not coagulate during the sterilization treatment. Each base contains 2% corn starch, ½% salt and 4% non-fat milk solids. The numbers on each curve refer to the manufacturing data for that milk given in table 1.

Whey protein was substituted for casein containing milk products in those instances in which coagulation was a problem. The whey protein, after coagulation, remained softer and more finely dispersed than did casein. Whey protein was generally added to the mix as whey cream containing about 12 per cent fat. This cream was separated from fresh sweet cheese whey. Cream sauce suitable for the preparation of creamed peas, mush-

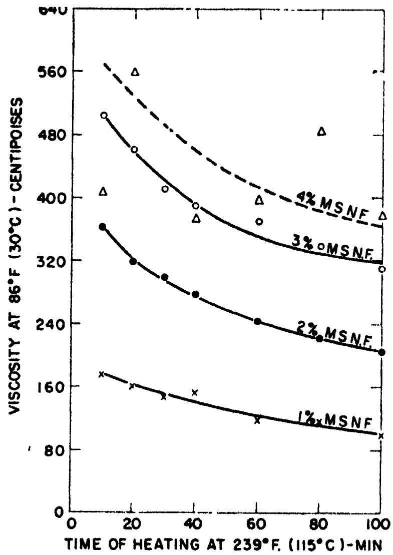

FIG. 4. The effect of increasing quantities of milk-solids-not-fat (M.S.N.F.) upon the viscosity of creamed bases sterilized for different periods of time. The complete mixes were heated to 149° F. and homogenized at 2500 pounds pressure before the usual heating to 176° F. and canning. All mixes coagulated during the first few minutes of sterilization. Mixes containing 4% M.S.N.F. were very badly coagulated, hence the broken line indicating the approximate location of this curve. Skim milk #1 (table 1) and corn starch #1 (fig. 1) were used. Each mix contained 4% butteroil, 2% starch and 1% salt in addition to the skim milk.

rooms or similar products contained 8 per cent to 12 per cent fat derived from whey cream, 3 per cent to 5 per cent starch or flour and 1 per cent salt. The sauce with the whole or chopped vegetables could be sterilized at 240° F. for as long as 90 minutes without serious damage to the body of the product. This sauce was homogeneous and in good condition after one year storage at room temperature. Plain, condensed, or dried sweet cheese

TABLE 4

The effect of the addition of an inert phase upon the heat-stability of skim milk. Fresh skim milk was diluted with an aqueous suspension of the inert phase. The final concentrations were milk solids not fat, 6%, and inert phase 1%

Inert phase added to milk	Heat-stability of milk at 239° F.
None—control	193
Tomato pulp, centrifugally separated, washed and adjusted to same reaction as milk	160
Filter paper, ground in 95% alcohol for 35 hours in a ball mill, filtered, washed, boiled in water, added to milk	152

whey was found to be an excellent source of non-fat whey solids for canned food products.

A plain but heavy-bodied white sauce was made which withstood storage at room temperature for several years with little change. This preparation contained 94 per cent whole milk by weight, 5 per cent flour, and 1 per cent salt. The mix was heated to 185° F. (85° C.) with constant stirring, canned and sterilized at 239° F. for 25 minutes. A gel structure was built up by the casein and the flour. The sterilization period was not long enough to allow the casein to gather into aggregates and expel whey.

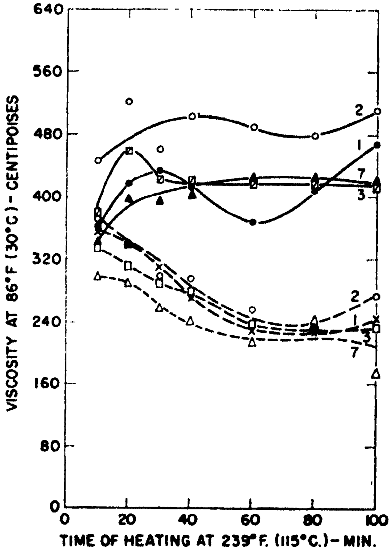

FIG. 5. The effect of agitation during sterilization upon the viscosity of creamed mixes made from different samples of dried skim milk. The complete mixes were heated to 149° F. and homogenized at 2500 pounds pressure before the usual heating to 176° F. and canning. The solid lines indicate samples sterilized without agitation, while the samples represented by the broken lines were sterilized with the reel running at 4 r.p.m. The number on each curve refers to the milk of table 1 which was used in the mix. The casein of all mixes was coagulated before the 10-minute sterilization period was reached. Each mix contained: dry skim milk—3%, butteroil—3%, corn starch #1—2%, salt—1%.

DISCUSSION

Dried milk and dried whey of various types were used in the manufacture of cream soups and similar foods. Preliminary experiments showed that the heat stability of spray-dried milk was not significantly changed by the drying process. But the heat-treatment previous to spraying largely determined the heat-stability of the powder. High-temperature forewarming was found by Webb and Holm (3) to increase the heat-stability of milk only when it was concentrated after forewarming to a solids-not-fat content greater than 12 per cent. The skim milks of table 1 which received the most drastic heating were the least stable when reconstituted to 9 per cent solids. Fresh milk, cream and whey or the concentrated forms of these products were well adapted to use in cream-style foods. Their heat-stabilities were governed by processing steps commonly employed in the concentrated milk industry. However, the heat-stability characteristics of a milk product were important only in the simpler mixtures. Coagulation was unavoidable in cream soups and other products which contained destabilizing ingredients.

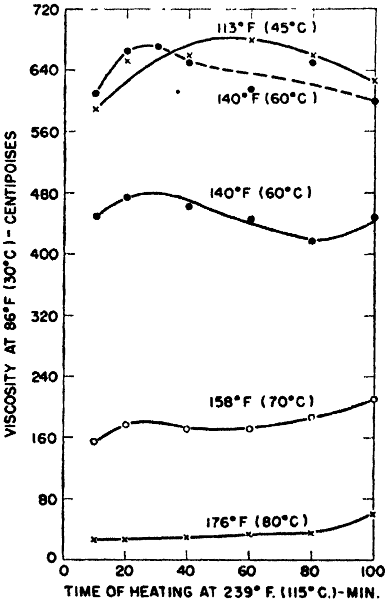

FIG. 6. The effect of homogenization at different temperatures upon the viscosity of creamed mixtures. Each mix except the one represented by the broken line was heated to the temperature designated on the curve and homogenized at 2500 pounds pressure. The starch and salt of the mix indicated by the broken line were added after homogenization. Before canning, all mixes were heated to 176° F. The sterilizer reel was held still during sterilization. Visible curd formed in all samples during the first 10 minutes of sterilization. The composition of the mixes was: dried skim milk (#1, table 1)—3%, butteroil—3%, corn starch (#1, fig. 1)—2%, salt—1%.

The problem was generally one of controlling the kind of curd which formed rather than in preventing its formation.

A processing temperature of 239° F. (115° C.) was used. Substantially the same results in terms of heat-stability and body development were obtained at 239° F. as were found in half the holding time at 250° F. (121.1° C). Temperatures higher than 239° F. were sometimes used for bacteriological reasons. For information on processing requirements for non-acid foods, reference may be made to the work of the National Canners Association (2).

In the preparation of the mixes it was found necessary to heat them to at least 176° F. (80° C.) with stirring before putting them into cans. This allowed the starch to swell and become evenly distributed in the mix and made a more homogeneous product. However, homogenization of the swollen starch granules caused severe destabilization of the casein during sterilization. For this reason the mixture containing starch was best homogenized at about 113° F. (45° C.). When a higher pre-homogenization temperature was used, the starch was added after homogenization.

Homogenization was useful in reducing the size of the fat globules and of the particles of fiber and pulp in the mix. The resulting improvement in the dispersion of the inert components of the product assisted in the development of a smooth body. The casein, when provided with innumerable nuclei, including those furnished by the starch binder, coagulated in a finely divided condition. The presence of a well-dispersed, inert material lowered heat-stability, but the formation of a uniformly smooth coagulum was ample compensation for this added instability.

Viscosity development in milk-starch-salt mixtures followed a pattern similar to that shown by evaporated milk during sterilization (1). Maximum thickening occurred at the coagulation point or shortly thereafter. Ideal conditions were reached when the heat stability of the product was equal to the heating time required to sterilize it. But the destabilizing nature of the ingredients of cream-style foods caused coagulation early in the sterilization period. When the sterilization process was continued beyond the thickening phase, moisture was expressed by the casein. A watery and lumpy body was produced by this moisture, and by the tendency of the casein to gather into firm aggregates. This condition was avoided by the use of a starch or flour binder, which was responsible, with the casein, for building body. The binder took up the moisture expressed by the casein as coagulation proceeded. The quantity of binder needed was dependent upon the percentage of casein present, the extent of its coagulation, and the amount of other water-holding constituents in the mix. Approximately 1 part by weight, of starch or flour binder was used for 2 parts of milk-solids-not-fat, but this ratio varied between 1:1.3 and 1:3.0.

SUMMARY

1. The use of milk products and their relation to the problems of coagulation and viscosity development in canned, sterilized cream-style foods including cream soups and sauces were studied. Milk constituents contributed to the flavor and nutritive value of the products and increased their viscosity.

2. The ingredients used in sterilized milk foods caused rapid coagulation of the milk protein in these mixtures. Factors which contributed to the production of a smooth, heavy-bodied product were: use of optimum quanti-

ties of starch and flour binders to take up moisture expressed by the casein, comminution of inert pulp and fibrous material by homogenization, sterilization with a minimum of agitation during the period when a coagulum was forming, maintaining maximum heat stability characteristics in the milk ingredients and substitution of whey protein for milk protein when coagulation of the casein in a product was excessive.

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CONTROLLED EXPERIMENTS ON THE VALUE OF SUPPLEMENTARY VITAMINS FOR YOUNG DAIRY CALVES

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Feeding of supplementary vitamins to young calves has recently attracted considerable attention. Since the publications on this subject by the Wisconsin workers (1, 2), some commercial concerns have sold vitamin preparations as a panacea for many types of calf losses. The true value, however, of extra vitamins to the young calf when added to a normal diet needed further study. Therefore, a series of experiments was begun early in 1942 to test the possible value of supplementary vitamins for very young calves under conditions in New York State.

EXPERIMENTAL

All calves in these experiments were allowed to remain with their dams for at least 24 hours and were fed their dams' milk for approximately 5 days after birth. This procedure insured an initial fill of colostrum, which has been shown by many workers to be beneficial in the early nutrition of the calf (4). Each calf was kept in a separate pen and was fed individually. Each calf was fed a limited amount of whole milk, according to the Cornell dry calf-starter system (5), and was weaned after receiving 350 pounds of milk over a period of 7 or 10 weeks depending upon the breed. Ayrshires and Holsteins were weaned at 7 weeks of age; Guernseys and Jerseys, at 10 weeks of age. Dry calf-starter and hay were supplied the calves at approximately 2 weeks of age. No skim milk whatever was fed.

Experiment I

In March, 1942, a preliminary trial was conducted with 5 Holstein heifer calves. Calves exhibiting varying degrees of scours were fed a gelatin capsule 3 times weekly. Each capsule contained the following vitamins: 48,000 U.S.P. units of vitamin A in oil, 1,000 units of vitamin D (irradiated yeast), 2.33 mg. of thiamin chloride, 2.33 mg. of riboflavin, 11.66 mg. of calcium pantothenate, 11.66 mg. of choline chloride, and 23.33 mg. of niacin.

Feeding the capsules produced no noticeable curative effect. The loose condition seemed to run a normal course before improvement occurred. There were no control calves in this preliminary study.

A more extensive controlled experiment with 18 purebred heifer calves was started in April, 1942. In one group of 9 calves, each calf received a gelatin capsule containing the above-listed vitamins at birth and three times

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TABLE 1

The effect of supplementary vitamins on the rate of growth and on the occurrence and severity of scours

Number	Breed	Date of birth	Body weight		Relation of age (weeks) to occurrence and severity of scours*							
			Birth	16 weeks	1	2	3	4	5	6	7	8
			lbs.	*lbs.*								
Supplementary vitamins												
1484	Ayrshire	5/10/42	68	182	+	++	+++	–	–	–	–	–
1489	Ayrshire	5/30/42	84	215	–	++	++	–	–	–	–	+++
1502	Ayrshire	7/14/42	80	194	–	++	+	–	–	–	–	–
1478	Guernsey	4/20/42			No scours (died at 5 days of age).							
1486	Guernsey	5/14/42	70	168	–	++	+	–	–	–	–	–
1500	Guernsey	7/12/42	55	148	–	+	–	–	–	–	–	–
1480	Holstein	4/21/42	88	210	–	–	++	–	–	–	–	–
1491	Holstein	6/26/42	92	255	–	–	–	–	–	–	–	–
1504	Jersey	7/15/42	45	146	–	–	+	–	–	–	–	–
Average (% of normal)			99.9	102.7								
Gain as % of normal				104.4								
No vitamin supplement												
1479	Ayrshire	4/20/42	68	175	–	+	++	–	–	–	–	–
1506	Ayrshire	7/20/42	90	192	–	–	–	–	–	–	–	–
1501	Guernsey	7/12/42	61	143	–	+	++	–	–	–	–	–
	Holstein	5/17/42			No scours (died at 5 days of age).							
1493	Holstein	7/1/42	93	249	–	–	–	–	–	–	–	–
1507	Holstein	8/1/42	92	264	–	+	–	–	–	–	–	–
1477	Jersey	4/5/42	45	126	+	–	++	–	–	–	–	–
1481	Jersey	4/21/42	55	141	–	–	+	+	–	–	–	–
1485	Jersey	5/10/42	44	155	–	+	+	–	–	–	–	–
Average (% of normal)			98.8	100.3								
Gain as % of normal				101.4								

* – = No scours, + = Mild, ++ = Moderate, +++ = Severe.

a week thereafter until one month of age. A second group, serving as controls, was given the same care and management, but received no supplementary vitamins. Careful daily observations were made of the incidence and severity of scours and of the rate of recovery. Most of the calves received a dry calf-starter containing no animal protein, and were fed a second cutting Ladino clover hay of excellent quality. These results are shown in table 1.

One calf in each group died from unknown causes. Neither of these calves showed any indication of having scours. There was no evidence (table 1) that feeding supplementary vitamins at this level had any beneficial effect on the health of the experimental calves. The frequency of occurrence, severity, or duration of a scouring condition appeared to be unaffected by the addition of extra vitamins to the normal diet. Most of the cases of scours were treated by the administration of either sulfathiazole, sulfanilamide, or sulfapyridine. No severe cases of scours were encountered.

Up to 16 weeks of age, there was little difference in the rate of growth between the two groups of calves (table 1). The calves receiving supplementary vitamins had an average birth weight of 99.9 per cent of Ragsdale's normal (3) and showed an average gain from birth to 16 weeks of age of 104.4 per cent of normal. The control calves at birth averaged 98.8 per cent of normal weight and had an average gain up to 16 weeks of age that was 101.4 per cent of normal. This difference in gain was considered to be of little or no practical importance. It might easily be attributed to the number of Jerseys in the group since the Jerseys in the college herd as a rule are of a smaller type than those used in establishing Ragsdale's normal. At 16 weeks of age, there were no apparent differences in the physical condition or appearance of the two groups of calves. The Holstein calves appeared to experience somewhat less scouring than the other breeds although the numbers studied were too small to warrant a conclusion.

Experiment II

A second experiment was initiated in October, 1944, involving the use of 24 purebred Holstein heifers from the college herd. The calves were selected at random to serve as controls or to receive supplementary vitamins from birth to one month of age. One capsule was given daily during this period. Each capsule contained the following vitamins: 6,000 U.S.P. units of vitamin A, 1,000 U.S.P. units of vitamin D, 250 mg. of vitamin C, 100 mg. of niacin, 50 mg. of vitamin E, 10 mg. of riboflavin, 10 mg. of pyridoxine, 10 mg. of thiamin, 50 mg. of calcium pantothenate, and 1,000 mg. of choline. This combination of vitamins was fed to insure that most of the possible vitamin deficiencies would be met. It was felt that if beneficial results were obtained from this mixture, later work could be undertaken to determine the most effective vitamin combination.

The control group of calves received the same feed and management, but did not receive the supplement of vitamins. The results of this experiment are shown in table 2.

TABLE 2

The effect of vitamins on the incidence, severity, and duration of scours and the rate of growth in Holstein calves

Number	Date of birth	Body weight		Relation of age (weeks) and scours*							
		Birth	20 wks.	1	2	3	4	5	6	7	8
				Supplementary vitamins†							
		lbs.	*lbs.*								
1720	11/ 8/44	98	248	–	–	–	–	++	+	–	–
1721	10/20/44	105	302	–	+	+	+++	+++	++	+	–
1729	12/25/44	75	277	–	+	–	–	–	–	–	–
1732	12/29/44	78	249	+	++	–	+++	+++	++	++	–
1734	1/ 3/45	81	244	+	+	–	–	–	–	–	–
1745	2/11/45	84	305	–	–	–	–	–	–	–	–
1747	3/ 1/45	107	295	–	–	–	++	+	+	+	+
A1	3/17/45	70	248	++	+++	+	–	–	–	–	–
1749	3/18/45	82	264	++	+++	++	–	–	–	–	–
1756	4/ 1/45	96	290	–	++	+	–	–	–	–	–
1763	5/ 3/45	104	301	–	–	–	–	–	–	–	–
1769	5/18/45	102	311	–	–	++	–	–	–	–	–
Average		90.2	277.8								
Average (% of normal)		100.2	100.9								
Total gain (% of normal)			101.2								
				No vitamin supplement							
1718	10/20/44	92	228	–	–	–	+	–	+	–	–
1723	11/25/44	106	293	+	+	+	+	+	–	–	–
1724	12/12/44	90	286	–	–	–	–	+	–	–	–
1730	12/28/44	90	328	–	–	–	–	–	–	–	–
1731	12/29/44	95	293	–	–	–	–	–	–	–	–
1736	1/19/45	98	265	–	–	+	–	+	–	–	–
1742	2/ 1/45	93	314	+	–	–	–	–	–	–	–
1751	3/25/45	88	290	–	–	–	–	–	–	–	–
1752	3/20/45	98	269	–	–	+	+++	+	+	+	+
1758	4/ 4/45	87	290	–	–	–	–	–	–	–	–
1760	4/12/45	91	241	+++	+++	+	–	–	–	++	++
1765	5/10/45	85	304	–	–	–	–	–	–	–	–
Average		92.8	283.4								
Average (% of normal)		103.1	102.9								
Total gain (% of normal)			102.8								

* – = No scours, + = Mild, ++ = Moderate, +++ = Severe.

† These vitamins were supplied by Hoffmann-La Roche, Inc., Nutley 10, N. J.

From these data it is evident that under the conditions of the experiment, supplementary vitamins were of no value in preventing scours. In the group that received supplementary vitamins, only 2 calves failed to show some looseness during the first 8 weeks. Six of the 12 calves in the control group receiving no added vitamins showed no evidence of scours during the same period. The duration of scours or the severity of the condition seemed to be unaffected by the supplemental vitamin feeding. In fact, in this experiment there were more frequent cases and more severe scours in the group that received supplementary vitamin capsules than in the control group.

Figure 1 shows the growth of the two groups of calves from birth to 20 weeks of age. It is apparent that supplementary vitamin feeding in the amounts used in this experiment had no effect on gain in weight. At birth, the control group and the group receiving supplementary vitamins weighed 103.1 per cent and 100.2 per cent of Ragsdale's normal, respectively. At 20 weeks of age, the body-weight relationship was nearly the same; the control group, 102.9 per cent, and the group receiving added vitamins, 100.9 per cent of normal for Holstein calves at that age. Over the 20 weeks, the control group and the vitamin supplemented group gained at approximately the same rate, 102.8 per cent and 101.2 per cent of Ragsdale's normal, respectively.

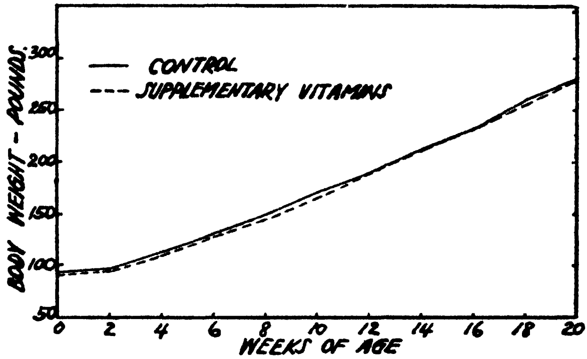

FIG. 1. The average birth weights and average weekly body weights of 12 Holstein heifer calves receiving supplementary vitamins and 12 Holsteins receiving no added vitamins.

From the amount of fleshing, the condition of the hair coat, and the general appearance of the calves, it was impossible to distinguish the calves that had received supplementary vitamins from those that had received none.

Experiment III

A third experiment was set up in a Holstein herd maintained under somewhat less favorable feeding conditions. The calves were divided at random into three groups and were fed one tablet daily from birth to 30 days of age containing either the complete vitamin mixture used in the second experiment, no vitamins, or a tablet containing vitamin A, 5,000 U.S.P. units; vitamin D, 1,000 U.S.P. units; ascorbic acid, 250 mg.; and niacin, 100 mg. In general, the feeding practice and management were similar to that described above.

The results of this test, summarized in table 3, again show the ineffectiveness of vitamin supplements in preventing scours in calves. As an average, the calves fed the vitamins gained somewhat more in weight during the first 8 weeks, but the difference is not significant, statistically.

TABLE 3

The effect of supplementary vitamins upon the growth rate and the health of Holstein heifer calves

Number	Date of birth	Body weight		Relation of age to occurrence and severity of scours							
		Birth	8 wks.	1	2	3	4	5	6	7	8
				No vitamin supplement							
		lbs.	*lbs.*								
103	11/ 6/44	116	158	–	–	–	–	–	–	–	–
106	12/ 6/44	81	140	–	–	–	++	++	++	–	–
110	3/18/45	99	140	–	–	–	–	–	–	–	–
112	4/ 8/45	76	104	–	++	–	–	–	–	–	–
115	4/28/45	88	120	–	++	–	–	–	–	–	–
Average		92.0	132.4								
Average (% of normal)		102.2	93.1								
Total gain (% of normal)			77.4								
				Complete vitamin capsules*							
104	11/15/44	92	170	–	–	–	–	–	–	–	–
105	11/15/44	74	130	–	–	–	–	–	–	–	–
111	3/27/45	75	118	–	–	–	–	–	–	–	–
113	4/16/45	86	118	++	+++	+++	+	–	–	–	–
114	4/19/45	80	116	–	–	++	–	–	–	–	–
Average		81.4	130.4								
Average (% of normal)		90.4	91.7								
Total gain (% of normal)			93.9								
				Vitamins A, D, C, and niacin*							
107	12/ 6/44	90	130	–	–	–	–	–	–	–	–
108	12/ 7/44	84	135	–	–	–	–	–	–	–	–
109	12/23/44	99	144	–	–	+++	–	–	–	–	–
116	6/ 5/45	68	112	–	–	–	–	–	–	–	–
117	7/30/45	93	150	–	–	–	–	–	–	–	–
Average		86.8	134.2								
Average (% of normal)		96.4	94.4								
Total gain (% of normal)			90.8								

* These vitamins were supplied by Hoffmann-La Roche, Inc., Nutley 10, N. J.

DISCUSSION

The results of the tests reported show that over a three-year period, feeding extra vitamins to new-born calves did not prevent scours nor reduce the incidence, duration, or severity of scours. The vitamin supplements had no certain effect upon the growth rate or general appearance of the calves up to 16 or 20 weeks of age. Thus no justification was found for feeding extra vitamins in these tests. The writers are inclined to the view that the natural feeds furnished an adequate supply of the necessary vitamins for the growth and well-being of the experimental calves. Further investigations are needed to determine whether the same is generally true under average farm conditions in this area. This question is now being studied.

These results were based on close daily observations of the condition of the experimental calves. In some of the trials, choice second-cutting alfalfa hay was fed that should have insured a generous carotene intake in these cases. Acknowledging the possible laxative effect of alfalfa hay, it was observed that the calves showed no less tendency to scour than calves that received hay of a lower carotene potency.

It should be recognized that the calves received a limited amount of whole milk during 7 or 10 weeks following an initial feeding of colostrum. All calves received their dams' milk for 5 days after birth. Under conditions that exist on some farms, where whole milk is fed in greater amounts over a longer period and is finally replaced with skim milk, one might logically expect less benefit from adding extra vitamins to the diet than with less favorable feeding and management.

While it is known that a severe deficiency of vitamin A and possibly other vitamins will cause diarrhea, care must be exercised in assuming that scours in young calves is a definite indication of vitamin deficiency. It is difficult, if not impossible, to determine whether a particular case of scours is due to nutritional deficiency, specific bacterial infection, or faulty feeding and management practices of which there may be many.

SUMMARY

Studies are reported involving approximately sixty heifer calves of various dairy breeds, largely Holsteins, which show that supplementing a normal diet with capsules or tablets containing vitamins A, D, and E, ascorbic acid, and several of the B-vitamins did not reduce the incidence or severity of scours. Extra vitamins in the amounts fed had no certain effect on the rate of growth or the general appearance of the calves during the early months of life. Under the feeding and management conditions involved, there was no advantage to calves from adding extra vitamins to the normal ration.

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THE LOSS OF NUTRIENTS IN HAY AND MEADOW CROP SILAGE DURING STORAGE*

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Livestock men appreciate the value of having adequate amounts of good quality roughage for feeding between pasture seasons. All too often the quality of the material coming out of storage, either from the mow or silo, fails to come up to expectation. The losses in quality are generally quite obvious and include changes in color, aroma, physical condition, and palatability. Other losses in quantity of total dry matter, digestible nutrients and carotene may also have taken place. Although these are just as real as the visible losses in quality, they are not generally recognized. Of course, the ideal sought for in storage is to obtain a palatable feed with a minimum loss of dry matter and nutrients. The purpose of this review is to present the information concerning the losses taking place during storage of meadow crops under different conditions and treatments in the mow and silo.

STORING AS UNDERCURED HAY

Spontaneous combustion may result from storing undercured material in the mow. This results not only in a total loss of the stored material, but frequently in a loss of the building and sometimes in a loss of the surrounding buildings and their contents. It is estimated (48) that the farm losses from this cause in the United States amount to $20,000,000 annually. Different theories have been advanced regarding the nature and causes of the various processes involved in spontaneous combustion. These have been reviewed by Browne (11) and more recently by Henson (32). Investigators agree that a certain amount of moisture is necessary to start the processes leading to this trouble. Roethe (71) believes that the losses can be reduced if the hay is stacked (or put in the mow) with a moisture content not to exceed 30 per cent. Likewise, Henson (32) considers such hay safe from excessive heating. For hay put into stacks in England, Firth and Stuckey (19) recommend that the moisture be 25 per cent or less. Watson (83) points out that there is danger from relatively wet patches in the hay as put into storage or subsequently caused by a leaky roof or by drainage. Experience has shown that hay wet from floods is subject to spontaneous combustion (11).

Black and various shades of brown hay may also result from storing undercured hay. Swanson and co-workers (80) put up a stack of approximately 40 tons of alfalfa containing 59.42 per cent moisture. On opening the stack it was found that several different kinds of hay had resulted, including black, two shades of brown, mouldy green, and a small amount of apparently good hay. However, the loss of organic matter had amounted to 39 per cent. The loss of nitrogen-free extract was calculated at 45 per cent and of protein at 33 per cent. A large portion of the hay was unfit for feed. Steers fed the black hay made unsatisfactory gains.

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Hoffman and Bradshaw (38) show losses in brown hay of 22 per cent dry matter. The fats, sugars, and hemicellulose groups were especially affected, resulting in a hay of very inferior quality.

In some recently reported work by Bechtel and associates (6), analyses are given for a "normal" hay taken from the outside of a stack, brown hay from the inner portion of the stack, and a black hay, coarsely chopped, selected from the remains of a partially-burned stack on a nearby farm. The analyses for the three kinds of hay are given in table 1. The analyses of the "normal" and brown hays are similar, but those for the black hay are markedly different, especially in respect to crude fiber and nitrogen-free extract. The loss of nitrogen-free extract in storage in the brown hay was 15 per cent and in the black hay 39 per cent more than the "normal" hay.

TABLE 1

Chemical composition of normal, brown, and black hays used in digestion trials

Description	Percentage composition of dry matter							
	Moisture	Ash	Crude protein	Crude fiber	N-free extract	Ether extract	Calcium	Phosphorus
Normal	20.4	9.72	21.51	25.20	41.16	2.40	1.53	0.29
Brown	11.4	10.60	21.04	28.17	38.37	1.93	1.56	0.30
Black	16.9	11.66	21.03	35.03	30.42	1.85	1.57	0.28

In a feeding trial the normal hay was found to be superior to the brown hay. The black hay was not fed in a comparative milk-production trial. The results obtained in feeding these hays indicated that the normal hay was adequate in feeding value, whereas the brown and black hays were decidedly inadequate.

The digestion coefficients for the three hays were determined and are shown in table 2.

Table 2 shows that, with exception of ether extract, the digestibility coefficients were lower in the brown and black hays than in the "normal" hay. The extremely low digestibility coefficient for protein in the black hay is in agreement with previous work as reported by Watson (83). The brown and black hays proved unpalatable.

Reed (70) also reports that brown hays lacked palatability. In his work, Holstein cows normally eating 17.9 pounds of a No. 2 Michigan hay consumed only 14.4 pounds of a brown hay. He encountered dry matter losses of 33.7 per cent and 49.5 per cent in producing the brown hay.

There is some disagreement in the findings on palatability and feeding value of brown hay as compared to "normal" hay. Maynard and coworkers (49) report brown alfalfa to be more palatable and slightly higher in feeding value for fattening lambs than a comparable green alfalfa. Grassman, as reported by Henson (32), found that dairy cows ate more of a brown hay than of a normal hay but that the digestibility of the hay was lowered according to the degree of browning. In some specially conducted palatability trials, Willard (89) found that alfalfa browned in the stack had retained its palatability, although the sugar content of the hay had been reduced by the browning process. The Kansas Station (15, 16), reports that dairy cows had to learn to eat brown hay. The results of two feeding trails showed 4.3 per cent less milk and 4.1 per cent less fat on the brown hay than on a green hay. Henson (32) and Folger (20) believe that the inferior feeding value of brown hay may be masked under practical feeding conditions by a greater consumption of such hay.

TABLE 2

Digestibility percentages and palatability comparisons of normal, brown, and black hays

	Normal hay	Brown hay	Black hay
Digestibility percentages:			
Dry matter	60	41	27
Crude protein	67	16	3
Crude fiber	41	36	14
Nitrogen-free extract	72	59	53
Ether extract	25	33	42
Palatability:			
Amount eaten per 1,000 lb. liveweight	20	15	10

As an explanation for this lack of agreement on "brown hay," there is reason to believe that considerable variation exists between different lots of "brown hay" and the processes by which they were produced. Thus, Fred and Peterson (23) produced "brown hay" by wilting alfalfa to 30 to 40 per cent moisture content and then packing it tightly in a silo under a heavy top seal. The resulting material had a brownish-green color and a pleasant odor and was readily eaten by cattle. The dry-matter loss was reported as only 5 per cent.

NORMAL HAY

"Well-cured" hay or that containing less than 30 per cent moisture also undergoes some changes in storage. Woodward and Shepherd (95) stored two 5-ton lots of hay containing 27 to 25 per cent moisture, respectively. When removed from storage approximately 3 months later, the moisture content had dropped to 9.5 per cent and 8.5 per cent, with total dry weight losses of 4.1 and 5.3 per cent, respectively. Repeating this work (74) with

TABLE 3

*Analyses of first-cutting alfalfa, as put into and taken out of the mow, with percentage losses of nutrients (dry basis)**

	Dry matter	Crude protein	Crude fiber	N-free extract	Ether extract
	%	%	%	%	%
Into mow	75.1	16.7	29.3	44.5	1.87
Out of mow	87.7	17.4	32.5	39.8	1.84
Losses, per cent	6.9	3.3	+ 3.1	16.8	8.90

* Vermont Bul. 494, table 4.

a drier hay (15.8 per cent moisture) gave a dry matter loss of 5.33 per cent. The authors state that this "consisted principally of nitrogen-free extract, with small losses of crude protein and ash and no losses of crude fiber or fat." Very similar losses, 3 to 8 per cent, in total dry weight of eight different hays stored with 12 to 28 per cent moisture were found by Camburn and associates (12, 13). The losses occurred principally in the nitrogen-free extract. Table 3 is taken from the tables presented by these workers on the analyses of hay going into and coming out of the mow.

A similar study has been made by Hodgson and associates (35) at the Washington Station. The authors describe the weather conditions as humid. Their work represents three successive years, with mows containing from 51 to 68 tons of hay. The losses were more extensive than those shown above from the Vermont Station. These data are summarized in table 3A.

Chopping hay into the mow requires that the moisture content be lower than when the forage is stored as long hay, in order to prevent excessive

TABLE 3A

*Weighted average percentage of first and second cuttings of hay made from grass and clover forage as put into the mow and as taken out, with the percentage loss of dry matter and nutrients sustained during the storage period for three different years**

	Dry matter	Crude protein	Crude fiber	N-free extract	Ether extract
1943 season	%	%	%	%	%
Into mow	73.0	7.9	31.9	52.1	1.5
Out of mow	83.3	9.2	32.8	48.8	1.8
Losses, per cent†	26.4	13.9	24.3	31.0	14.1
1935 season					
Into mow	78.2	7.9	28.9	55.5	2.1
Out of mow	78.2	7.8	31.5	52.8	1.9
Losses, per cent	8.9	10.0	0.6	13.3	17.4
1936 season					
Into mow	78.2	9.0	33.4	48.4	1.9
Out of mow	80.2	9.8	32.2	49.6	1.5
Losses, per cent	12.9	4.8	16.0	10.6	31.1

* Washington Bul. 366, tables 5, 6, and 8 (dry basis).

† Included in this loss was 12,272 pounds of hay discarded because it was musty and unfit to feed, which accounts for the unusually high losses for this year.

heating. At a given moisture content, chopped hay will heat more than long hay (51). As an important item in storage, chopped hay occupies one-half, or less, of the storage space of long hay. These points are illustrated in the work of Woodward and Shepherd (95, 74), as summarized in table 4.

Feeding trials with milking cows indicated a higher value for the long hay than for the coarsely chopped, brown, or finely chopped hay containing some black. This latter hay lacked palatability. In hay stored with the lower moisture content, there was little difference between long and chopped hay in feeding value. The advantages of chopped hay over long hay lie in the mechanics of handling and storing and in a reduction in feeding wastage. There appears to be no nutritional advantage from chopping (10, 44, 57, 90). Likewise, there is no nutritional benefit from grinding hay; in fact, in some cases this has been shown to lower digestibility (21, 29).

TABLE 4

Comparison of long and chopped hay in storage

	Chopped ¼-in. hay	Long hay	Chopped ¾-in. hay	Long hay	Chopped ¾-in. hay	Long hay
Moisture when stored, %	27.0	27.4	25.3	25.3	15.8	15.8
Maximum temperature in storage, degrees F.	151	122	128	120	106	81
Loss of dry matter, %	6.5	4.1	3.2	3.5	4.9	5.3
Color out of storage	Brown–Black	1–25* green	Light brown	14–45* green	47–48* green	54–57* green
Storage space per ton, cu. ft.	152	475	218	475	227	592
Carotene preservation						
Carotene into storage, p.p.m.	46	46	74	74	75	75
Carotene out of storage, p.p.m.	0.9	2.9	3.9	3.9	20.7	31.9

* Refers to the range in percentage of green color as compared to that in the greenest commercially produced hay.

Salting of hay may check bacterial growth and delay, but not inhibit, mold development according to the laboratory tests conducted by Stuart and James (79). In storage studies Henson (32) found salt "markedly beneficial" in one test, slightly beneficial in a second, and without effect in six other tests. He does not favor the general use of salt but believes that losses from excessive heating in under-cured hay may occasionally be reduced or prevented by the use of salt.

The work on this question conducted by the Bureau of Chemistry and Soils of the United States Department of Agriculture has been summarized by Roethe (71) as follows: "Although salt in liberal quantities may retard fermentation, there is no experimental evidence in the Bureau's possession that the addition of salt to hay as it is stored, at least in amounts safe for feeding, will prevent spontaneous ignition." Shutt (75) has compared additions of 10, 20, and 30 pounds of salt per ton of hay to no treatments in stacked clover-timothy hay. No outstanding differences were shown by chemical analyses. The salted hay had a better aroma, was less dusty, had more color, and was also more palatable than the unsalted hay. The 30-pound addition contained too much salt for satisfactory feeding and proved "very loosening on the steers."

Field baling of cured hay containing less than 25 per cent moisture may be practiced with good results, if precautions are taken to provide ventilation when the material is stored, according to studies conducted by Henson (32). He believes that the temperatures developed in hay bales correspond with those of mowed hay of the same moisture content.

Watson (83) presents data on the composition, digestibility, and quality of hay baled at moisture contents varying from 16 to 40 per cent. The most suitable moisture level was 20 to 24 per cent; below this there was a loss of color due to bleaching and above this range, brown patches occurred, due to fermentation and molds and dustiness were encountered. Miller and associates (52) report that hay containing from 24 per cent to 40 per cent moisture when baled molded when piled under conditions of adequate natural ventilation.

TABLE 5

Analyses of a first-cutting, artificially dried alfalfa hay as put into and taken out of storage. (Calculated to 90 per cent dry matter basis)†*

	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
Into mow	6.59	14.09	25.90	41.38	2.02
Out of mow	6.79	13.91	28.78	38.67	1.85
Percentage change	+0.20	− 0.18	+ 2.88	− 2.71	−0.17

* Loss of total dry matter, 1.99 per cent.

† Vermont Bul. 494, table 4.

Artificially dried hays (kiln dried) would be expected to show only very small losses in organic matter in storage, because of their low moisture content. The analyses presented by Camburn and associates (12), in which the average loss amounted to 1 per cent, justifies such a belief. An illustration of their analyses on an artificially dried, first-cutting alfalfa going into and coming out of the mow is shown in table 5.

With the exception of a few instances in which the drying has been admittedly inefficient, the results obtained in feeding artificially dried hays and grasses have been satisfactory (3, 4, 14, 46). The digestibility (ether extract excepted (33)) and palatability are also high (27, 33). Hodgson and associates (36) have shown that grass could be dried at exhaust gas temperatures up to 350° F. without lowering the digestibility of the material. At 400° F. the digestibility of the protein was markedly decreased.

In fact, the artificially dried material has been shown to have the same value as the green growing crop (62).

Artificially dried hays may at times be deficient in their vitamin D content (5), although this is not always true (25). Wallis (82) has shown that there may be marked differences in the vitamin D content of even sun-cured hays.

Since the process of artificial drying is essentially one of curing rather than of storing, it does not come within the scope of the present review. However, from the viewpoint of preserving nutrients, artificial drying comes close to being the ideal. From a practical viewpoint the cost of the drying operations and the lack of availability of equipment for the average farmer preclude the widespread farm adoption of this process.

Mow drying by forced ventilation may supply the means of overcoming some of the objections to artificial drying. Weaver and Wylie (87) believe that, in order to operate the barn driers economically, 75 per cent of the water in excess of that normally in dry hay should be removed in the field. Barn-dried hay averaged 2.3 per cent more leaves and 19 per cent more carotene than field-cured hay. The barn-cured hay had a superior feeding value. Dexter and Sheldon (18) also report that, "As a whole, the hay was of excellent quality." They encountered musty hay in the upper 2 feet. They favor putting the hay into mow-drier at approximately 35 per cent moisture. From observations on five farms in Virginia using mow driers, Connelly (17) reports that the resulting hay has been of good quality. Increases in milk production are attributed to feeding this mow-dried hay as compared to feeding the ordinary sun-cured hay. Investigators (39, 41, 50, 72) in this field have been chiefly concerned with the engineering features involved. Input-output data on the hay seem to be lacking. However, it is reasonable to suppose that the percentage preservation under this system would be determined by the extent of natural heating involved.

To speed up the drying of the hay, Strait (78) tried heated air. He was able to reduce the loss of carotene to 18.5 per cent during the mow-drying process.

Carotene losses in stored hay present a serious problem. Carotene (pro-vitamin A), the form in which vitamin A exists in forage plants, is unstable. It is readily oxidized and is subject to photolysis (26). Hauge (28) has shown that enzymes in the plant are also capable of destroying carotene. In a later publication (85) of which Hauge was a joint author, the opinion is expressed that the enzymes may be active in dried hays. Recent work by Mills and Hart indicate that this is the case (53, 54). In hays that have been subjected to a special heat treatment such as in artificial drying which inactivates the enzymes, the retention of carotene should be higher than in sun-cured hays. This tendency is shown in the reports of Camburn and associates (12, 13). These authors give the progressive losses of carotene in sun-cured and artificially cured hays by 2-month intervals for 360 days. After 300 days, the percentage retention of carotene was 55 for the artificially dried and 27 for the sun-cured hay.

In the storage of naturally cured hay the losses of carotene are also influenced by the extent of heating which the hay undergoes, which in turn is influenced by the moisture percentage. Thus, in the work of Woodward and Shepherd (74, 95) as shown in table 4, the hay stored with 27 to 25 per cent moisture and which had heated extensively, lost practically all of its carotene, whereas the hay stored with 15.8 per cent moisture retained a fair amount of this substance.

Guilbert (26) presents data to show that temperature (*per se*) has an effect on carotene losses. When alfalfa hay and meals were stored in heavy paper containers in the dark for 8 weeks at a temperature ranging from 23° to 41° F. there were no losses of carotene, but at 68° to 86° F., the loss was 30 per cent and at 154° to 176° F., the losses ranged from 62 to 87 per cent. More recent work by Wilder and Bethke (88) confirms these findings. Kane and associates (43) estimate a 3 per cent loss in carotene per month for baled hay in winter storage and a 6.5 per cent loss for storage in warmer weather. For summer storage the losses ranged from 11 to 21 per cent. Smith (76) also notes a seasonal effect in the alfalfa stored in bales. In samples of baled hay as found on the market, evidence of a caked or set condition or of a tobacco, sweated, or ground odor may be taken as an indication of a decidedly low carotene content (91). After comparing the colors of different hays with their carotene content, these authors conclude that "no definite quantitative relation between these factors can be expected." Work (43) in the same laboratory had previously shown that the percentage loss of carotene was more rapid than the loss of green color.

STORING GREEN FORAGES AS SILAGE

Losses in the silo come chiefly from top spoilage, the seepage of juices through the bottom or sides of the silo, and from fermentation. In addition, there may be some material at the extreme bottom of the silo that is unfit for feed and hence lost. Another loss, which is only partial, is generally encountered at the top of the silo following the removal of the top spoilage. This silage, although feedable, does not have the quality or palatability of the silage further down. In experimental work this is generally fed off before starting the experiment so that the silage will be of "uniform quality." In practical feeding, farmers have difficulty in getting their animals to eat a sufficient amount of this intermediate material. In some regions partial losses and unsatisfactory feeding results may be caused by the silage freezing.

Top spoilage on a percentage basis varies with the amount of silage or depth of the silo, according to Newlander and associates (59). They give estimates of top spoilage of 6.1 per cent for a 24-foot silo and 3.3 per cent for a 36-foot silo, as based on their experimental work. Nevens (58) shows that the amount of top spoilage can be materially reduced by applying a proper seal. Briefly, his plan is to provide an air-tight cover, such as roofing material and then add a sufficient weight of material to pack the silage near the surface. It seems reasonable to believe that an adequate top seal, in addition to reducing top spoilage, may also lessen the amount of "indifferent" silage beneath the spoilage.

Seepage losses have recently been reported by Archibald and Gunness (2) for a 100-ton wooden silo over a 7-year period. The losses in dry matter from the seepage have averaged 0.54 per cent, with a range from 1.08 to 0.12 per cent. The authors express the following opinion concerning this "loss"; "The improved quality of the silage when the juice is permitted to drain off will largely offset any small losses." No carotene was found in the juice. Peterson and associates (67) report a loss of 1.77 per cent dry matter in seepage from 17 tons of alfalfa ensiled by the A.I.V. process. The New Jersey Station (1) reports losses ranging from 2 to 18 per cent of the total weight ensiled. The dry matter percentage of the seepage ranged from 0.19 to 1.7 per cent. There was a greater loss with molasses than with phosphoric acid–treated silage.

Otis (63) presents seepage data from a 14 × 45-foot silo, especially equipped for such a study. One hundred and twenty-two tons of green alfalfa with a moisture content of 72.3 per cent were treated with phosphoric acid. The loss of dry matter by way of the seepage was found to be 1.6 per cent of the total dry weight of the ensiled material. The juice was found to contain 93.4 per cent water, 3.2 per cent crude protein, 2.0 per cent mineral ash, and 1.4 per cent nitrogen-free extract.

Fermentation losses as reported for silage may be too high where the oven-drying method of moisture determination has been used. This is the claim of Perkins (65), and also of King (45), who find that volatile products are driven off and accounted for as moisture in oven-drying. Perkins (65) recommends a toluene distillation method with corrections for the amount of volatile acids.

The losses (top spoilage excluded) in total dry matter, protein and carotene in a large number of silages made from various crops and with various treatments have been determined by Woodward and Shepherd (96). The summary of their results is presented in table 6.

The results were obtained largely with silos of one-ton capacity, which the authors believe yielded silage comparable to that from large silos. The dry-matter losses ranged from 5 to 10 per cent, top spoilage excluded; and protein losses from 2.8 to 6.6 per cent. In respect to palatability, the high-dry-matter silages, either wilted or treated with molasses, rated high; whereas the silages treated with mineral acids were low in palatability. The authors discourage the use of hydrochloric and sulfuric acids for silage preservation, regardless of the successful preservation of feed nutrients. They state: "The silage is definitely unpalatable." Special emphasis is

TABLE 6

*Dry matter, protein and carotene losses in grass silage, with palatability data**

Experimental difference	Moisture	Dry matter eaten†	Losses in the silo exclusive of top spoilage		
			Dry matter	Protein	Carotene
	%	*lb.*	%	%	%
Higher moisture	70.6	21.4	10.3	4.8	22.2
Lower moisture‡	45.0	24.8	8.3	2.8	38.3
Higher pH(4.76)	62.6	22.9	8.7	7.6	30.5
Lower pH (4.36) by molasses§	61.5	24.2	8.6	7.8	23.5
Higher pH(5.18)	66.8	23.6	7.7	6.6	26.2
Lower pH(3.66) by 2 N acids‖	67.9	16.3	5.0	2.9	9.3

* Taken from Woodward and Shepherd (96) tables 1, 2 and 3.
† Dry matter consumed per cow per day.
‡ By wilting or added dry matter. Average of 25 comparisons.
§ Various additions of molasses. Average of 19 comparisons.
‖ By additions of mineral acids.

placed upon the proper moisture content as a factor in making good silage. Similar conclusions have been reached by Hayden (30) and coworkers.

Taylor and associates (81) conclude that a loss of 10 to 15 per cent of the total dry matter is nominal and unavoidable in ensiling. Likewise, from an extensive series of tests the Vermont workers (12, 13, 60) show an average "intangible" loss of 11.5 per cent.

The feed analyses of the silages indicate that the silage is not materially different from the crop going into the silo. To illustrate this point, sample analyses taken from three different investigations and representing three different silage processing methods are shown in table 7.

TABLE 7

Comparison of analyses of crops going into and coming out of the silo

	Dry matter	Crude protein	Crude fiber	N-free extract	Ether extract
Clover—treated with 3 per cent molasses (actual composition basis)*					
In-going clover	32.56	3.59	10.21	15.63	0.77
Out-going edible silage	31.67	3.84	10.44	14.09	0.88
Mixed grasses—treated with phosphoric acid (actual composition)†					
Mixed grasses	29.30	3.30	9.10	13.90	0.80
Grass silage	26.80	3.00	8.60	12.40	0.80
Grass—untreated (dry matter basis)‡					
Material ensiled	28.90	11.80	23.70	54.60	3.30
Good silage (recovered)	26.90	12.60	27.70	45.50	4.40

* Vermont Bul. 494 (12).
† New Jersey Bul. 683 (81).
‡ Washington Bul. 348 (34).

The digestibility coefficients of the various feed constituents in grass silage were found to be approximately equal to those in the original grass, in trials conducted by French (24). Crude protein was less digestible in the silage than in the original grass—49 per cent as compared to 60 per cent. The ether extract was 10 per cent more digestible in the silage than in the original grass. That the ensiling process does not necessarily depress the digestibility of crude protein is indicated in the results obtained by several other investigators (22, 34, 61). Although these investigators have not compared the digestibility of the silages with the original material, the digestion coefficient for crude protein is higher than that found by French (24). Excessive heating as the cause of lowering the digestibility of protein in silage is suggested by Woodman (92), who obtained a digestibility coefficient of 12.2 per cent for crude protein in a stack silage that had heated. Watson and Ferguson (84) report a coefficient of 35.3 for the protein of a stack silage in which heating had occurred as compared to 67.7 for that of a tower ensilage in which little heating was noted.

Carotene preservation in meadow-crop silage has been shown to be high. In fact, in some cases the carotene values for silages have been reported as higher than the original material (31, 47, 60). These high values were shown by Quackenbush and associates (69) to have been caused by the presence of pigments produced by the action of the silage acids on xanthophyll. A method for separating the pigments has been worked out by Peterson, Hughes and Freeman (68), and further modified by Moore (56).

Camburn and associates (12) present carotene analyses on several different silages at the end of 8 months in the silo. The percentage retention of carotene for the different treatments was as follows: 57 with phosphoric acid treatment, 52 with no molasses, 47 with 8 per cent molasses, 29 with 2–4 per cent molasses. In another trial (13) these investigators obtained the following percentage retentions: 78 with A.I.V.-treated, 43 with phosphoric acid, 22 with molasses-treated and 18 with no treatment.

In the results obtained by Woodward and Shepherd (96), presented in table 6, the carotene retentions were higher than those indicated above. The silage preserved by the A.I.V. method headed the list. Carotene preservation was also shown to be high in the investigations of Taylor and associates (81), who state that "grasses or legumes, ensiled with acids or molasses contain two or three times as much carotene as does corn silage."

After an extensive series of trials including such treatments as wilting, phosphoric acid, salt, and corn-and-cob meal, Stone and co-workers (77) state, "The highest carotene values were usually obtained in alfalfa ensiled with molasses." This differs from the Vermont findings (12, 13) which favored the A.I.V. treatment. In all treatments a satisfactory fraction of the original carotene was preserved according to the work of Stone and associates (77).

Milk and butterfat produced from meadow-crop silages have been shown to contain increased amounts of carotene and vitamin A. Thus, with A.I.V. silage Peterson and associates (66) found 50 per cent more carotene and 40 per cent more vitamin A in the milk than when the ordinary well-balanced winter ration was fed. Bechdel and co-workers (7) also mentioned an increase in carotene content of the butterfat produced on the high-carotene legume silages. At the Purdue Station the feeding of alfalfa-brome grass silage treated with molasses was found to increase the carotene and vitamin A content over that produced on corn silage (86). Hodgson and associates (37) also indicate substantial increases in the vitamin A potency of butterfat from feeding grass silage. This same observation has been made repeatedly at the Ohio Station (30).

The "grass-juice" factor which has been shown to be present in grass silage is also transferred to the milk (42). Silage prepared with phosphoric acid contained somewhat more of the factor than molasses-treated and untreated silages. An alfalfa silage containing a soured-whey concentrate showed an excellent preservation of this factor. The "grass-juice" factor is so named because of its abundance in young grass. It is an unidentified water-soluble substance of a growth-promoting nature for rats and guinea pigs.

The viability of weed seeds as affected by the siloing process, although indirectly related to the subject of this review, has a practical significance in farming methods. Woodward (93) shows that the germinating powers of the seeds from a number of different weeds are destroyed in the silo.

The type of silo appears to be relatively unimportant in respect to the nutrient preservation. In the work reviewed several different kinds of silos have been used. Bohstedt (9) states, "The type of silo is important only in so far as it excludes air and provides means for compression of the silage."

MOISTURE EVALUATION AND CONTROL NEEDED IN STORING FORAGES

Probably the one single factor that calls for the most consideration in putting crops into either the mow or silo is simply the moisture content of the material. On most farms moisture is "determined" by the "feel," coupled with experience. Woodward (94) has described a mechanical device for estimating the moisture in crops intended for the silo. Another method based on the reaction of calcium carbide with moisture has been presented by Parks (64). Or, if electricity is available, the rapid-drying method (55) of blowing heated air through a sample can be used. This has been shown to agree within one per cent of the laboratory oven-drying method (77). With an adequate moisture control the losses in storage can be reduced to a minimum.

ROUGHAGE REVIEWS

Articles covering the general subject of roughage including the work on storage, have been published by Bohstedt (9), Huffman (40), LeClerc (48), Watson (83), and Woodward *et al.* (97). The subject of barn hay-driers has been reviewed by Schaller, Mitchell, and Dickerson (73) and the subject of grass silage by Bender and Bosshardt (8).

SUMMARY

Storing as hay. In "well-cured" long hay or that containing less than 30 per cent moisture, the dry-matter loss in storage will vary from 3.5 to 9 per cent and in some cases more. This loss occurs principally in the nitrogen-free extract and to a lesser degree in the ether extract.

Hay containing in excess of 30 per cent moisture is not considered safe for ordinary storage. In such cases, extensive heating results that may lead to either spontaneous combustion or to the production of charred, blackened or browned hay. Dry matter losses depend upon temperatures attained and their duration. In browned and blackened hay such losses may range from 15 to 40 per cent. Heating reduces the digestibility of the hays, especially of the proteins, and increases the carotene loss. Although some browned hays have been found palatable, in general, browned hay, and blackened hay especially, are less palatable than hays that have not heated.

At a given moisture content chopped hay will heat more than long hay. A "long chop" is preferable to a "short chop." Such hay should contain less than 25 per cent moisture for storage.

Salting hay will not prevent excessive heating in under-cured forage.

For field baling, hay should contain less than 25 per cent moisture.

The losses in dry matter and nutrients (carotene excepted) of artificially dried hay are practically negligible in storage.

Mow-drying of hay by forced ventilation with or without artificial heat has been shown to result generally in hay of good quality with a high feeding value. In cases where fermentations and heating have occurred, it is logical to assume that losses will be encountered just as in ordinary storage. Much more work on the nutrient content, feeding values and palatability of mow-cured hay needs to be done.

Losses in carotene take place in the storage of "well-cured" long hay in the mow, so that at the end of 300 days, such hays may have only one-quarter of their original value. In the heating of hay the carotene may be entirely lost. The losses are greater in warm weather than in cold weather.

Storing as silage. The percentage loss from top spoilage will vary with the size of the silo and the precautions taken in sealing the silo. A loss of 3 to 6 per cent of the total dry matter of ensiled material is a general estimate. The actual weight of spoiled material removed from the top of a silo may represent a proportionally larger amount of original forage, because of the loss of organic matter through decay and the drying out of the material.

The percentage losses through seepage also vary with the size of the silo and the moisture content of material ensiled. Normally the dry matter losses from this cause are less than 1 per cent, when the original forage contained 70 per cent or less moisture.

Fermentation losses in dry matter will normally vary between 5 and 10 per cent. This is exclusive of losses from top spoilage or seepage.

The feed analyses of silage is similar to the analyses of the material ensiled. The digestibility of the silage is likewise similar to the original green material, except in silage in which extensive heating has taken place. In such cases the digestibility of the protein is lowered.

Carotene is generally well preserved in the ensiling process. According to some work the carotene retained in the silage averaged from 60 to 90 per cent of that ensiled. Other investigators show lower carotene retentions. Considerable variation exists in the amount of carotene retained in silage. The type of treatment has been shown to have an effect on carotene preservation. In general, treatment with mineral acids shows high retention and no treatment or wilting has given the lowest retention.

The high carotene content of the silage is reflected in the carotene content of milk produced on such silage.

The "grass-juice factor" has been shown to be present in grass silage, especially in silage preserved with soured whey or with phosphoric acid.

The ensiling process destroys the germinating powers of a number of different weed seeds.

Proper moisture evaluation and control are needed for minimizing the losses in storing forages as hay and silages.

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ANNOUNCEMENT

ANNUAL MEETING, IOWA STATE COLLEGE

June 18–20, 1946

REGISTRATION AND HOUSING

Registration headquarters will be in the Memorial Union, Iowa State College.

Housing facilities will be available in college dormitories and local hotels. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

PROJECTION EQUIPMENT

Lanterns will be available, upon request, for projection of standard and 2″ × 2″ slides. Projectors for 8 and 16 mm. movies also will be available by arrangement. Requests for projection equipment should be made at the time abstracts of papers are submitted to the respective section chairmen.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section committee meetings should contact Elmer Hansen. F. E. Nelson will assign rooms for meetings of committees of the Manufacturing Section.

SPECIAL MEETINGS

Rooms for a limited number of breakfasts, luncheons or dinners for special groups will be available at the Union. Shortage of help will not permit table service, so the groups will have to go through the cafeteria lines and thence to the reserved rooms. Requests for such reservations should be made to F. E. Nelson, Dairy Industry Department, Iowa State College, Ames, Iowa.

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LIVE WEIGHT VERSUS METABOLIC BODY SIZE IN DAIRY COWS AND GOATS

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Kleiber and Mead (12) have recently published in this Journal a vigorous[1] critique of the FCM_8/W_1 philosophy. (FCM_8 is milk-energy yield for the first 8 months of lactation, W_1 is live weight within 31 days after parturition of the same lactation, both FCM_8 and W_1 to be measured at each lactation of each cow or goat.) See also their (11) earlier better-tempered critique on the same subject. In regard to the metabolic body size philosophy they state: "This theory, suggested by Kleiber, was tested and confirmed by Brody and his co-workers." The reference here is presumably to Kleiber's (8) masterly paper on body size and metabolism, and his (9) later paper on size of animal and food utilization.

WHAT IS METABOLIC BODY SIZE?

The following two paragraphs are quoted from Kleiber (10):

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[1] In some respects their paper shows more vigor than perception. Thus, in their criticism of a short article (3) they dwell on an incidental point in the article and apparently fail to perceive the specifically stated major point, namely that when the equation, $FCM = aW^b$, is used to describe the regression of milk-energy yield on live weight the value of b varies greatly according to the stage of lactation at which live weight is measured (cf. 2, table 1). They present their California data without stating the stage of lactation at which live weight was measured. The FCM_8/W_1 philosophy accepts W_1 as a proper and sufficient measure of size of the cow or goat. It is, of course, their right not to accept that view but failure to designate the stage of lactation at which live weight was measured in their data indicates lack of perception as regards a very important detail. The article (3) in question is based on 11 records and as they rightly contend (and as is recognized in the article) the considerable inherent and environmental variability in milk-energy yield as between cows and lactations renders unreliable (but by no means necessarily false) for general application the fact that in these 11 records FCM_8 is, by the fitted equation, proportional to the 1.07 power of W_1. A similar criticism does not apply to the main comparison for the simple reason that it deals with the same milk-energy yields, only the stage of lactation at which live weight was measured being variable. In general Kleiber and Mead's point concerning variability in milk-energy yield, as between cows, is very important. Of coordinate significance is the variability as between lactation of the same cow, the import of which is not generally and fully appreciated.

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> . . . An animal may be thought of as a group of equal size-parts. These parts are not thought of as structural elements such as cells or moleculars but as abstract units. By analogy with the engineers' method of measuring motors in number of horse power, biologists can express the size of animals in units of power e.g. as kilocalories of standard metabolism per day which may be abbreviated to ''Calsad''. . . .
>
> . . . Since the standard metabolism in kilocalories of various animals from rat to steer can be predicted as 72 times the $\frac{3}{4}$ power of body weight in kilograms the number of Calsads in an animal is proportional to the $\frac{3}{4}$ power of its body weight. . . .

From this it appears that metabolic body size is predicted standard metabolism or estimated basal metabolism, and kg.$^{\frac{3}{4}}$ is estimated basal metabolism per day in units of 72 kilocalories. Metabolic body size is not size in the sense of weight.[2] It is size in the sense of metabolism, under the conditions

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[2] Brody in his recent invaluable volume uses the term ''metabolically effective body weight'' (1, p. 383) which he defines as proportional to the 7/10 power of live weight. He states explicitly that ''. . . a 1200-lb. cow cannot produce ten-fold the milk energy of a 120-lb. goat at its upper limit, and a 1400-lb. cow cannot produce twice the milk energy of a 700-lb. cow at its upper limit, because large animals have relatively larger supporting structures and, therefore, relatively smaller visceral organs and areas than small animals,'' (1, p. 856). He explains, by dimensional analysis, that for animals to possess stability the supporting structures must be proportional to the 3/2 power of live weight, or approximately so.

Bone is a typical supporting structure. Mitchell, Card, and Hamilton (13) have published experimental data on live weight and weight of bone in chickens during the course of growth as per the following figures:

Cockrels									
W, gm.	232	449	673	993	1361	1786	2236	2583	3253
Bone, %	16.8	16.0	16.2	18.6	17.9	18.5	18.0	16.2	15.7
Capons									
W, gm.					1375	1702	2285	2684	3188
Bone, %					17.3	18.7	17.1	16.4	15.6
Pullets									
W, gm.				961	1342	1842	2340		
Bone, %				16.8	16.7	14.5	14.2		

These data indicate no tendency for bone percentage to increase with live weight within sex. But according to Brody's explanation bone percentage must be approximately proportional to $W^{\frac{1}{2}}$. By direct observation bone percentage in a 4-lb. pullet is 15. According to Brody, bone percentage in a 1600-lb. cow would have to be 300 (that is, $15 \times 1600^{\frac{1}{2}}/4^{\frac{1}{2}} = 300$). This absurdity raises a question about the validity of his explanation. His metabolically effective body weight is not weight, but, like Kleiber's metabolic body size, is simply predicted basal metabolism.

When we fit the equation, $Y = aW^b$, to a set of observed Y's and W's we systematically distort W by the exponent b so that W^b is proportional to Y, as near as may be. We must think of W^b not as an actual measurement in units of weight, but as a predicted measurement in units of Y (Y = metabolism in calories, lactating maintenance in lb. *DN*, milk-energy yield in lb. *FCM*, or the like). Thus, to say that kg.$^{\frac{3}{4}}$ is the unit of metabolic body size is to say that predicted basal metabolism in kilocalories per 20 minutes is the unit of metabolic body size. If we talk in terms of W^b we must not think in terms of weight. In general, in the fitted equation, $Y = aX^b$, ''units'' of X^b are units of estimated Y, the unit of estimated Y being a times the original unit of measurement of Y. In the sense of quantitative science, X^b, where b is not unity, has no units except in terms of estimated Y.

prescribed for the measurement of basal metabolism, predicted from live weight by means of an interspecies equation.

Table 1 provides an easy means of finding the numerical value of kg.$^{3/4}$, where live weight is given in lb., no fractions. Table 1 is presented as superior in design and execution as compared with Kleiber and Mead's (12) table 2. As to execution, among the 101 entries of kg.$^{3/4}$ in their table 2, 13 are erroneous, namely at 680, 740, 870, 950, 990, 1330, 1400, 1430, 1460, 1490, 1520, 1550, 1580 lb. (In a later edition of their table, Hilgardia, vol. 16, p. 571, the error at 1400 lb. has been corrected.) In magnitude the errors are minor, but inexcusable because the relation 1 kg. = 2.2046 lb. is established and the kg.$^{3/4}$ relation is purely a matter of computation. As to design; their table is formulated by use of uniform intervals of 10 in lb., while the present table is formulated by use of uniform intervals of 1 in the values of kg.$^{3/4}$. The present design provides finer numerical distinctions than the design of their table. However, if live weight is given in 10-lb. units, no fractions, their table is handier to use (first, of course, correcting the 13 errors mentioned).

TABLE 1

Table for reading metabolic body size, in kg.$^{3/4}$ to closest whole number, where live weight is given in lb., no fractions

	0	1	2	3	4	5	6	7	8	9
1	45	51	58	64	71	78	86	93	101	108
2	116	124	132	141	149	157	166	175	183	192
3	201	211	220	229	239	248	258	267	277	287
4	297	307	317	327	338	348	359	369	380	390
5	401	412	423	434	445	456	467	478	490	501
6	513	524	536	547	559	571	583	594	606	618
7	630	643	655	667	679	692	704	716	729	742
8	754	767	780	792	805	818	831	844	857	870
9	883	896	909	923	936	949	963	976	990	1003
10	1017	1031	1044	1058	1072	1086	1100	1113	1127	1141
11	1155	1170	1184	1198	1212	1226	1241	1255	1269	1284
12	1298	1313	1327	1342	1356	1371	1386	1400	1415	1430
13	1445	1460	1475	1490	1505	1520	1535	1550	1565	1580
14	1596	1611	1626	1641	1657	1672	1688	1703	1719	1734
15	1750	1765	1781	1797	1812	1828	1844	1860	1876	1892
16	1907	1923	1939	1955	1972	1988	2004	2020	2036	2052
17	2069	2085	2101	2117	2134	2150	2167	2183	2200	2216

To use, find the largest tabular value which is not larger than the given live weight and read metabolic body size from the corresponding marginal figures at side and top of table. An efficient procedure is to arrange the *W*'s in numerical order and divide the array into blocks at the division points shown by the table. Each of the *W*'s in a given block then has the metabolic body size value indicated. For example, *W*'s 1400–1414 inclusive each give metabolic body size 127.

WHAT IS LIVE WEIGHT?

The FCM_8/W_1 philosophy regards live weight as a directly proportional measure of the amount of protoplasm or work-stuff in the lactating cow or goat. This view is warranted by the data of Mitchell, Kammlade, and Hamilton (14) showing the weight of protein to be 13.6 per cent of live weight as an average of 6 shorn sheep (average live weight 77 lb.): and by the data of Trowbridge, Moulton, and Haigh (17) showing the weight of protein to be 14.2 per cent of live weight, as an average of 3 cows (average live weight 975 lb.). It seems permissible to say that the amount of protoplasm or work-stuff tends to be proportional to live weight of the two species. Live weight is, accordingly, estimated amount of protoplasm (work-stuff) in lactating cows and goats.[3]

An essential feature of the FCM_8/W_1 philosophy is that live weight at each lactation be measured shortly after parturition, that is, within 31 days (in accommodation to the system of monthly visits by the tester). Morgan and Davis (15) have published valuable detailed data showing the course of live weight with the course of gestation and lactation. (See 1, p. 431 for a graphic abstract of their data.) Clearly, the stage of the reproductive cycle at which live weight is measured should be prescribed: arbitrarily, if necessary; logically and usefully, if possible. W_1 is used in the FCM_8/W_1 philosophy in preference to W at some other stage for several reasons. Live weight may be regarded as a mixed measurement representing "pure size" plus fatness. It is well known that fatness at calving has a considerable influence on milk-energy yield. In a qualitative way that influence is properly recognized by dealing with FCM_8/W_1. Fatness is not properly recognized qualitatively by dealing with W at later stages. In addition to the logic of the case may be cited the experimental observation (5, p. 1041) that FCM_8/W_1 is independent of fatness at calving. It may be too much to say that a pound of fatness has the same influence on milk-energy yield as a pound of "pure size," but from the data available it appears the fatness influence is not less than that of "pure size," pound for pound.

FCM_8/W_1 VERSUS CAL.$_{10}$/METABOLIC BODY SIZE

Both these philosophies recognize milk energy as a proper biological measure of the quantity of milk yield. Both are expressed as an average per day, one in lb. 4 per cent milk for the 8-month partial lactation, the other in kilocalories for the 10-month partial lactation. Practically, calories may be estimated as 1 lb. *FCM* = 340 kcal., hence the difference narrows to the length of partial lactation used.

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[3] The experimental observations are not as direct or extensive as could be desired but the nature of the data lends confidence in the view that the weight of protein in lactating cows or goats is about 14 per cent of live weight. It is quite clear that the amount of protein does not follow Kleiber's ¾-power rule as between cows and goats. If a 975-lb. animal is 14.2 per cent protein, a 77-lb. animal would be 26.8 per cent protein by the ¾-power rule. The observations cited would not fail to detect a difference of that magnitude.

[4] A large part of the body protein is represented by the muscle tissues, which are only indirectly involved in the work of lactation. The question arises, is the amount of protoplasm directly involved in the work of lactation proportional to live weight as between cows and goats or as between individuals within species?

Official records started off with a 7-day period and later swung to the other extreme of a 365-day period. Popular favor is now with a 305-day period. The 305-day yield, as between individuals is much less affected by the inevitable variability in length of service period (parturition to next conception) than is the 365-day period. In this respect a further advantage is gained by shortening the period to 243 days (8 months) and the FCM_8/W_1 philosophy simply utilizes that biological improvement.[4]

AN IMPORTANT DIFFERENCE

A very important difference in the two philosophies is that one deals with milk-energy yield per unit live weight (live weight measured within 31 days after parturition, without age distinctions), while the other deals with milk-energy yield per unit basal metabolism (basal metabolism estimated as proportional to the $\frac{3}{4}$ power of live weight, presumably average live weight for the 10-month period, with age confined to given lactations, such as first lactations.)[5] The one measures the metabolism of lactation (milk-energy yield) in terms of the amount of work-stuff (protoplasm) in the goat or cow; the other measures the metabolism of lactation in terms of the whole metabolism of the goat or cow under conditions of starvation and the lowest possible level of work. Figure 1 attempts to show graphically, in a diagrammatic way, the nature of the difference in the two philosophies.

EXPERIMENTAL EVIDENCE

Figure 1 may be considered as presenting two alternative postulates. The final test of any postulate is its conformity with the evidence of experimental observations. What experimental evidence is available bearing on the validity of the postulates?

In lactating cows and goats the direct and indirect work of milk secretion precludes the direct determination of basal metabolism because the condition of lowest possible level of work cannot be provided during lactation.

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[4] Ordinarily yields are estimated from monthly 1-day determinations and FCM_8 is the average of 8 such determinations while FCM_{10} is the average of 10. Other things equal, an average of 10 is better than an average of 8, but study of individual lactation curves suggests that for biological comparisons, use of the 8-mouth period gains more by avoiding complications of advanced pregnancy than is lost by disregarding the ninth and tenth monthly tests. Compare figure 17 of Jensen *et al.* (7) and their finding that beyond 36 weeks after calving in the lactation curves of their critical Input-Output investigation, ". . . pregnancy rather than feed is the dominant factor in controlling milk production." For biological comparisons between individuals as to quantity of lactation, a rational procedure seems to be to simply side-step pregnancy complications by dealing with FCM_8. However the difference between FCM_8 and FCM_{10} is a minor rather than a major matter.

[5] Kleiber and Mead do not clearly define their philosophy with regard to these rather important details.

It is feasible to determine lactating maintenance as a function of live weight by straightforward statistical procedure, the principle of which was used by Wood and Capstick (18) many years ago. By such procedure, it has been shown (4) quite conclusively that as between cows and goats lactating maintenance is substantially proportional to metabolic body size, but between individuals within either one of the species lactating maintenance is proportional to live weight (4, fig. 3). That is, as concerns lactating maintenance in cows and in goats the two horizontal lines in figure 1 conform to the experimental evidence; the curved line (metabolic-body-size theory) does not conform to the experimental evidence. Unless the observations or

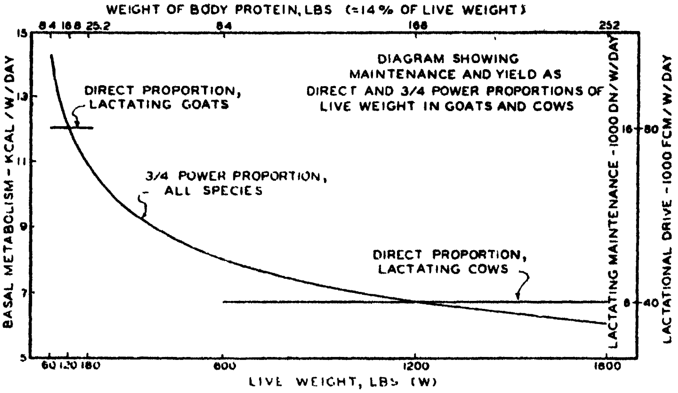

FIG. 1. Diagrammatic comparison of live weight and metabolic body size proportionality theories theories in goat and cow.

The regression lines represent the following equations:

Curved line, all species: basal metabolism in kcal./day = 72 kg.^{3}

Straight line, goats: lactating maintenance in lb. DN/day = 0.016 W

Straight line, cows: lactating maintenance in lb. DN/day = 0.008 W

Straight line, goats: milk-energy yield in lb. FCM/day = 0.08 W

Straight line, cows: milk-energy yield in lb. FCM/day = 0.04 W

Accepting the weight of body protein (scale at top of diagram) as proportional to live weight, and weight of protoplasm, or amount of work-stuff, as proportional to weight of protein, these relations may be expressed in terms of amount of protoplasm, or work-stuff, by merely changing the proportionality constant.

To make the comparison vivid, basal metabolism, lactating maintenance, milk-energy yield, are presented per unit live weight, plotted against live weight. Inclusion of mice weighing 0.025 lb. to 0.075 lb. makes the comparison still more striking. On the scale of the figure the basal metabolism line (curved line) would be practically vertical and indistinguishable from the ordinate at $W = 0$. At $W = 0.025$ basal metabolism per lb. live weight is 100; at $W = 0.05$ it is 84; at $W = 0.075$ it is 76. The corresponding horizontal line would be microscopic in length.

The impressive fact is the great difference in intensity of metabolism of the protoplasm, as between species, according to the mean size (live weight) of the species. Values of metabolism per unit live weight keep that difference before us; values of metabolism per unit metabolic body size conceal the difference from us.

method of analysis can be shown inadequate or invalid it must be concluded that the metabolic-body-size theory with respect to lactating maintenance within cows or within goats is false (but true as between the two species).

It may be legitimate to reason from the known relation between live weight and lactating maintenance in cows or in goats (that is, within species), to the unknown relation between live weight and basal metabolism within species during lactation. There is no need to take that step, however, because it is lactating maintenance and not basal metabolism that is of practical concern (in such matters as feeding standars, efficiency formulas and the like). On the theoretical side it is clear, for lactating cows and goats, that if basal metabolism, within species, is proportional to the $\frac{3}{4}$ power of live weight then lactating maintenance, within species is not proportional to basal metabolism.

The above weight-maintenance relations are based on average live weight, rather than W_1. For reasons above mentioned, it is desirable in considering the weight-yield relation to deal with W_1.

LACTATIONAL DRIVE

This appellation is one of Dr. Brody's apt expressions. The words seem to fit the extraordinary ability of dairy cows and goats to produce milk under the conditions provided on commercial dairy farms. This ability has been developed far beyond the point of maximum good with respect to natural species survival value, until it has become parasitic in nature, beneficial to man rather than the animal. The extraordinary yields require extraordinary energy transformations or levels of metabolism. It seems appropriate to designate the characteristic with a descriptive term.

Lactational drive is quantitatively measured by FCM_8/W_1, which is lactation metabolism per unit time per unit weight of live protoplasm (work-stuff), with fatness at calving recognized and discounted. As thus measured lactational drive is confirmed as a real characteristic of dairy cows by the demonstration of pure lines (Nebraska-herd data to be presented from that Station at a later date). It appears that lactational drive is real, objectively measurable as FCM_8/W_1, and highly important in the practical and technical aspects of milk production.

Relative lactation capacity, as used by Kleiber and Mead, is measured by kcal.$_{10}$/kg.$^{\frac{3}{4}}$, which is lactation metabolism per unit time per unit basal metabolism, with fatness not specially recognized as influencing milk yield. It is the ratio of metabolism during lactation to metabolism during starvation and lowest feasible, temporary, level of activity.

The two measures are very different in concept. They are, however, quite similar numerically. In fact Kleiber and Mead (12) generalized that within one herd "... one would need the results from more than 500 cows to show a barely significant difference (random probability 5 per cent) between production rate per unit weight and per unit of metabolic body size. . . ."

STATISTICAL SIGNIFICANCE

Davis *et al.* (2) found in 367 lactations of 147 Holstein cows, by the fitted equation, $FCM_8 = aW_1^b$, that FCM_8 is proportional to the 1.01 power of W_1. Kleiber and Mead's generalization, above quoted, would indicate that the numbers are far too small (147 cows whereas more than 500 are required) to give statistical significance to this result as contradicting their metabolic body size theory of milk-energy yield. The result is statistically significant, as shown by the following approach.

TABLE 2

Number of lactations required to show a statistically significant difference in the validity of the two postulates, FCM_8/W_1 is independent of W_1 and $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$ is independent of W_1, as indicated by selected cases

Number of lactations (n)	367	171	33
Number of cows	147	81	8
Breed	Holstein	Jersey	Holstein*
Mean W_1, lb.	1348	894	1423
Smallest W_1, lb.	934	596	1072
Largest W_1, lb.	1854	1180	1716
$FCM_8 = aW_1^b$, value of b	1.01	0.96	0.73
Mean 1000 FCM_8/W_1, lb./day†	39.1	34.5	42.1
Mean 100 $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$, lb. day†	43.0	34.1	46.6
Coefficient of variation in FCM_8	20.0	20.6	12.9
Coefficient of variation in FCM_8/W_1	16.0	16.4	10.2
Coefficient of variation in $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$	16.3	16.5	9.8
Regression of 1000 FCM_8/W_1 on W_1 (b)†	+ 0.0001	− 0.0022	− 0.0080
Standard error (SE_b)	± 0.0020	± 0.0039	± 0.0046
Regression of 100 $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$ on W_1 (b)†	+ 0.0075	+ 0.0084	− 0.0006
Standard error (SE_b)	± 0.0022	± 0.0039	± 0.0051
Probability for FCM_8/W_1‡	0.96	0.58	0.08
Probability for $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$‡	0.0006	0.03	0.90

* Pure line with respect to FCM_8/W_1, being all the daughters of the Holstein bull No. 111, each daughter with 2 or more lactations in the Nebraska herd. These 33 lactations are included in the preceding 367.

† Note: 1000 $FCM_8/W_1 = 100\ FCM_8/\mathrm{kg.}^{\frac{3}{4}}$ when $W_1 = 933$.

‡ Probability that zero regression might occur by chance, as judged by the particular set of experimental observations. The corresponding number of lactations, N, to give $P = 0.05$ (that is a random probability of 5 per cent) is approximated by the formula $N = 3.8\ (SE_b/b)^2 n$. (For $P = 0.01$ use 6.6 in place of 3.8.)

We have two postulates to be tested against the experimental observations: 1) FCM_8/W_1 is independent of W_1; 2) $FCM_8/\mathrm{kg.}^{\frac{3}{4}}$ is independent of W_1. (For present purposes, in the metabolic body size postulate, FCM_8 is used instead of FCM_{10}, and $\mathrm{kg.}^{\frac{3}{4}}$ is derived from W_1 by use of table1.) In this form the validity of each postulate can be readily tested. The test for postulate 1 is to determine, by least squares, the values of b and its standard error in the equation, $FCM_8/W_1 = a + bW_1$. If b is not more than 1.95 times as large as its standard error the postulate is valid within the 5 per cent statistical standard. A similar test for postulate 2 is afforded by the equation, $FCM_8/\mathrm{kg.}^{\frac{3}{4}} = a + bW_1$. Table 2 presents data of the 367 Holstein lactations, together with data of two other selected cases.

From table 2, as judged by these 367 lactations, the chances are 96 in 100 that postulate 1 is valid, but only 6 in 10,000 that postulate 2 is valid. The statistical distinction between the two postulates is unequivocal.

By the formula given in table 2 the number of lactations that would provide statistical distinction at the 5 per cent level would be $3.8\ (22/75)^2\ 367 = 120$. At the same average number of lactations per cow this would require 48 cows—quite a different matter than 500.

Table 2 presents similar data for 171 lactations of 81 Jersey cows with a similar outcome. At the 5 per cent level the metabolic body size postulate would be invalid with $3.8\ (39/84)^2\ 171 = 140$ lactations (66 cows).

We turn now in table 2 to a special group of 8 cows representing a pure line with respect to lactational drive, and whose 33 lactations correspond closely to the metabolic body size theory of milk-energy yield.[6] The chances are 90 in 100 that postulate 2 is valid and only 8 in 100 that postulate 1 is valid. While postulate 1 is not invalid by the 5 per cent standard, a small increase in numbers would make it so, namely $3.8\ (46/80)^2\ 33 = 41$. Thus, as judged by these 33 lactations, 10 cows with 41 lactations would be sufficient in number to give statistical distinction as to the validity of postulate 2 and invalidity of postulate 1. The low numbers are associated with a low variability in FCM_8/W_1 ($CV = 10.2$).

From the cases of table 2, the indications are that if CV in $FCM_8/W_1 = 16$, CV in $W_1 = 12$, and the regression of FCM_8/W_1 on W_1 is zero the regression of $FCM_8/\text{kg.}^{\frac{3}{4}}$ on W_1 will be statistically significant at the 5 per cent level with 50 cows having an average 2.5 lactations. Reduction of CV in FCM_8/W_1 or increase of CV in W_1 may easily give statistical significance at the 5 per cent level with 10 cows and 40 lactations.

If the regression of $FCM_8/\text{kg.}^{\frac{3}{4}}$ on W_1 is zero, the regression of FCM_8/W_1

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[6] This result is not to be construed as a general confirmation of the metabolic body size theory in pure lines, as indicated by the following data on the daughters of each of 5 bulls (the letter indicates the breed):

Bull. No.	*Daughters*	*Lactations*	*Mean W_1*	*Mean 1000 FCM_8/W_1*	*Regression of 1000 FCM_8/W_1 on W_1*
13A	5	19	1055	35.3 ± 0.89	+ 0.0064 ± 0.0085
15G	8	17	1057	25.0 ± 0.90	− 0.0060 ± 0.0082
1H	8	33	1423	42.1 ± 0.76	− 0.0080 ± 0.0046
6H	13	35	1324	40.2 ± 1.07	+ 0.0016 ± 0.0061
9J	9	27	897	35.4 ± 1.24	+ 0.0091 ± 0.0129

Each of the 5 daughter groups behaves as a pure line with respect to FCM_8/W_1 in that comparison of variance between daughters and variance within daughter gives an F value below the 5 per cent level of significance or intraclass correlation is not significant statistically. The simple average of the 5 regression coefficients is + 0.0006. That is, these 5 pure lines (3 different levels) on the whole support the FCM_8/W_1 philosophy and contradict the metabolic body size theory of milk-energy yield. The total number of lactations (131) is sufficint to make the result statistically significant.

on W_1 will be statistically significant in a similar way as in the preceding paragraph.

The present direct method of distinguishing statistically between the validity of the two postulates is quite feasible as concerns number of cows and lactations required and not subject to the practically prohibitive requirement of more than 500 cows as deduced by Kleiber and Mead in their generalization previously quoted. Their method is based on the difference in expected yield by the two postulates in comparison to the standard error of the difference. Results by one method do not contradict results by the other method because the two procedures do not measure the same thing. If FCM_8 proportional to W_1 and FCM_8 proportional to kg.$^{\frac{3}{4}}$ (metabolic body size) are regarded as two alternative working postulates as to the relation between live weight and milk-energy yield the present method of testing validity seems preferable to that of Kleiber and Mead.

EXPERIMENTAL EVIDENCE ON GOATS

Data from specific determinations of FCM_8 and W_1 are almost completely lacking for goats. The best data available are those of the London Dairy Show (16) which afford accurate information on live weight and milk-energy yield during a 48-hour period, occurring on the average and most frequently in the seventh month of lactation. For the whole body of 318 records *FCM* is proportional to the 0.91 power of live weight, by the fitted equation, $FCM = AW^b$. This relation may not correctly represent an unselected population because small goats had to compete on a par with large goats in absolute yield, which introduces a material bias in terms of FCM/W. That is, in terms of the competition a small goat would need a large FCM/W to equal a large goat with a small FCM/W. Bearing that bias in mind these records definitely contradict the metabolic body size theory of milk-energy yield in goats.

To carry out a comparison like that of table 2, the records (20) of 14 goats of the British breed are selected. In these 20 records the regression of 1000 FCM/W on W is $+0.002 \pm 0.075$ (probability of zero regression, 0.98) and the regression of 100 $FCM/\text{kg.}^{\frac{3}{4}}$ on W is $+0.064 \pm 0.049$ (probability of zero regression, 0.19). Postulate 1 is valid, but postulate 2 is not invalid by the 5 per cent standard. Judged by these 20 records postulate 2 would be invalid if the number of records was increased to $3.8\ (49/64)^2\ 20 = 45$. This outcome again indicates that statistical distinction of validity as between postulates 1 and 2 does not require anything like 500 cows or goats where one or the other postulate conforms closely to the observations.[7]

[7] In case 3 of table 2 a low number of lactations (41) was indicated as sufficient to distinguish at the 5 per cent level as between the two postulates, and this was associated with a low variability in FCM_8/W_1 ($CV = 10.2$). In these 20 records of goats of the British breed the low number (45) similarly required is associated not with low variability in FCM/W ($CV = 15.0$) but with high variability in live weight ($CV = 17.9$,

WEIGHT–YIELD RELATION BETWEEN AND WITHIN SPECIES

It has been noted above that lactating maintenance per unit live weight is distinctly higher (about 100 per cent higher) for goats than for cows, but within either one of the two species, it is independent of live weight. That is, in the diagram of figure 1 the two horizontal lines correctly represent the observations and these relations may be accepted as experimentally established with respect to lactating maintenance per unit live weight.[8]

With respect to milk-energy yield per unit live weight the case is similar to that with respect to lactating maintenance per unit live weight, provided FCM_8 is used as the measure of yield and W_1 is used as the measure of live weight. However, the weight–yield relation is subject to more inherent and environmental variability than is the weight–maintenance relation. Thus, in footnote 6 we have pure lines ranging in 1000 FCM_8/W_1 from 25.0 to 42.1. It is unlikely that there is any such inherent difference with respect to lactating maintenance per unit live weight among these same cows.

Variability in FCM_8/W_1, as between animals, limits the assignment of very definite values but it appears that, as a species average, FCM_8/W_1 is about twice as high in goats as in cows.[9] Like lactating maintenance per

range = 107–250 lb.). An important advantage of the FCM_8/W_1 philosophy is that FCM_8/W_1 is substantially independent of age. The influence of age on FCM_8, independent of W_1 is practically negligible (cf. 2, fig. 5; 5, fig. 6; and 6, p. 16). In the use of FCM_8/W_1 age may be disregarded, which is not only an advantageous simplification but also provides greater range in live weight. This greater range in live weight, as just pointed out for the above 20 goat records, contributes to solution of the weight-yield relation. Kleiber and Mead (11, 12) limit their California Holstein and Jersey data to first lactations and this limits both the number of lactations and the range of live weight.

[8] This statement is warranted by more than one thousand results from carefully conducted feeding trials which consistently show that lactating maintenance within cows or within goats is not proportional to predicted basal metabolism (metabolic body size, $kg.^{\frac{3}{4}}$), but is rather proportional to a power of live weight in excess of unity. Within cows or within goats the use of $kg.^{\frac{3}{4}}$ (basal metabolism) as a directly proportional measure of lactating maintenance is definitely discredited by the experimental observations. The following words of Benedict are quoted from Brody: ''Basal metabolism is one thing. Energy-food requirements for maintenance is quite a different thing . . .'' (1, p. 471). Evidently there is occasion to question the use of metabolic body size as a proportional measure of the energy needs for lactating maintenance as within cows and as within goats. By inference the question may be extended to include milk-energy yield. So far as concerns the present paper, the question is raised only as within cows and as within goats, not as between the two species. The weight-maintenance relation as concerns lactating cows and goats is distinctly different within species than it is between the two species (4, fig. 3). A similar statement seems to apply to the weight yield relation, although it is more variable and less definitely established.

[9] If the goat lives twice as fast and half as long as the cow the lifetime milk-energy yield and lactating maintenance needs would be proportional to their live weight by the FCM_8/W_1 philosophy. According to Rubner as cited by Brody (1, p. 679) each would generate 200,000 kcal. per kg. live weight during the life cycle. If ½ of this is milk energy, a 50-kg. goat would have a lifetime yield of 5,000,000 kcal. and a 500-kg. cow,

unit live weight, milk-energy yield per unit live weight (FCM_8/W_1) conforms to the metabolic body size theory as between cows and goats. Does it also so conform within cows and within goats? Is the relation correctly represented by the two discontinuous horizontal lines (FCM_8/W_1 philosophy) of figure 1, or is it correctly represented by the one continuous curved line (metabolic body size philosophy) of figure 1? The answer to the question must be found in the evidence of experimental observations. Adequate data on FCM_8 and W_1, are lacking for goats but there is a considerable amount of such data on cows.

Dealing with those sets of data on FCM_8 and W_1 where the number of observations is large enough to afford reliability in the statistical sense and expressing the relation in the form, $FCM_8 = aW_1^b$, we have the following experimental evidence:

Source	*Breed*	n	$\overline{W}_1$	$1000\ \overline{FCM_8/W_1}$	b
Neb. Sta.	G	77	960	30.6	0.43
do	A	131	1031	33.3	0.84
do	J	171	894	34.5	0.96
do	H	367	1348	39.1	1.01
Ill. DHIA	J	163	814	36.5	0.98
do	H	255	1203	24.8	1.02

The Nebraska data (2) are all within one herd (at Lincoln). The Illinois data (6) from several herds, are brought within one herd with respect to b by statistical treatment.

Of these 6 sets of data, 1164 lactations: 4 sets, 956 lactations, conform very closely to the FCM_8/W_1 philosophy; 1 set, 77 lactations, conforms to neither philosophy although more closely to metabolic body size than to FCM_8/W_1; and 1 set of 131 lactations, falls about half way between the two philosophies. Clearly the experimental evidence supports the FCM_8/W_1 philosophy very well and the metabolic body size philosophy very poorly. It is desirable, of course, to have further experimental observations but the above data will correct the possible impression created by Kleiber and Mead (12) that the FCM_8/W_1 philosophy depends for support entirely on the 11 lactations mentioned above in footnote 1.

If there is a species (cow and goat) difference in FCM_8/W_1, associated with species live weight, as pictured in figure 1, is there a similar breed difference within cows? More specifically, is FCM_8/W_1 greater for Jerseys than for Holsteins? Evidence of the Nebraska data answers, no. Evidence of the Illinois data answers, yes. Clearly more evidence is needed. Is there any Experiment Station (or other) herd of Jerseys to exceed the Nebraska

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50,000,000 kcal. If the life of the goat is 10 years, the yield would be 1470 lb. *FCM* per year; and if the life of the cow is 20 years, the yield would be 7350 lb. *FCM* per year. (Note: $1470/50 = 2 \times 7350/500$, or goat $= 2 \times$ cow.) These figures are presented not as being necessarily exact, but rather as representing a point of view.

Station Holstein performance of 1000 $FCM_8/W_1 = 39.1$, on a comparable basis, that is, all lactations of all cows over a term of 20 years? Kleiber and Mead's (11) data indicate such a possibility, even probability, for the California Station Jersey herd, but until the actual data are reported there is no way to be sure.[10]

SUMMARY

This article considers lactating maintenance and milk-energy yield, in cows and goats, as proportional to live weight versus proportional to metabolic body size ($kg.^{3/4}$).

It may be accepted as experimentally established that lactating maintenance per unit live weight as a species average is about twice as high in goats as in cows, or lactating maintenance is proportional to metabolic body size as between the two species. It is equally well established that the same relation does not hold within either one of the two species. Consistently, within species lactating maintenance is proportional to a power of live weight in excess (not significant) of unity.

The weight–yield relation is similar to the weight–maintenance relation, provided live weight is measured as W_1, age is not restricted, and milk-energy yield is measured as FCM_8. Within cows the weight–yield relation is more variable than the weight–maintenance relation, both inherently and environmentally. Validity of the two postulates as indicated by observed values of FCM_8 and W_1 is tested by determining, by least squares the value of b and its standard error in the equation, $FCM_8/W_1 = a + bW_1$ or $FCM_8/kg.^{3/4} = a + bW_1$. If one of the postulates conforms closely to the observations the other postulate will be invalid by the 5 per cent statistical standard with about 50 cows and 125 lactations in case of CV in $FCM_8/W_1 = 16$ and CV in $W_1 = 12$. These numbers may be easily reduced to 10 cows and 40 lactations if CV in FCM_8/W_1 is reduced or if CV in W_1 is increased.

Six sets of data on FCM_8 and W_1 are available on cows, each set with number of lactations large enough to give reliability in the statistical sense.

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[10] Brody (1, p. 857) continues his repeated citations of a "700-lb." Jersey cow with 1000 $FCM_{12}/W = 101.6$. Taken at face value this is a phenomenal performance. (From what we know about feed requirements of lactating cows at such enormous yields this 700-lb. Jersey must have consumed an average of about 35 lb. of grain per day for 365 consecutive days!) He uses the case to show the improbability, for example, that any 2000-lb. Holstein can ever attain 1000 $FCM_{12}/W = 101.6$. That is, dealing with the highest individual Jersey and the highest individual Holstein, the FCM_8/W_1 philosophy is shown to be untenable, as he sees it. Such extremes are of great interest but hardly rule out the need of comparing herd averages with respect to FCM_8/W_1 for the Jersey and Holstein breeds. Years ago Eckles developed a famous herd of Jerseys at the Missouri Station. How does the average FCM_8/W_1 for the Missouri Jerseys compare with the Nebraska Holsteins on the basis of all lactations of all cows? The average life spans of Holsteins and Jerseys are approximately equal and by Rubner's philosophy (footnote 9) it may be anticipated their average FCM_8/W_1's are also approximately equal. Adequate experimental observations must answer the question.

Four sets support the FCM_8/W_1 philosophy almost perfectly; one set supports neither one of the two philosophies, but the metabolic body size philosophy less poorly; one set falls about half way between the two philosophies. The weight of experimental evidence as between cows distinctly supports the philosophy that FCM_8 tends to be proportional to W_1; and distinctly contradicts the metabolic body size theory of milk-energy yield (FCM proportional to kg.$^{\frac{3}{4}}$).

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THE EFFECT OF ROUTE DELIVERY ON THE FLAVOR, RIBOFLAVIN, AND ASCORBIC ACID CONTENT OF MILK

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It has been known for some time that riboflavin and ascorbic acid are readily destroyed by the sun's rays, but only recently has attention been called to this problem in milk (7, 8, 9, 10, 11, 12). The photolability of riboflavin in milk was demonstrated by Williams and Cheldelin (11), who observed as much as 64 per cent loss of this vitamin when milk was boiled in a light room for 45 minutes and only a 5 per cent loss when it was boiled in the dark. More recently Stamberg and Theophilus (9) found as much as 80 per cent loss of riboflavin when quart-size flint glass bottles were exposed to direct sunlight for six hours, while the same milk exposed in brown glass and paper bottles lost less than 10 per cent of its riboflavin. In a more recent paper, Stamberg and Theophilus (10) observed as much as 40 per cent loss after two hours exposure in quart-size bottles, while brown glass and paper containers gave good protection. Holmes and Jones (7) exposed one-half pint bottles of milk to known intensities of sunlight and obtained losses of riboflavin up to 85 per cent in two hours. On a dark rainy day they found about 10 per cent loss in one hour. Their data, in general, show that the loss of riboflavin increases with the intensity of the sunshine. These workers further observed that little ascorbic acid remained after 30 minutes exposure to sunlight.

Naturally, important losses of these vitamins, such as demonstrated by these workers, are significant if milk is permitted to remain on the customer's doorstep for long periods of time. Furthermore, since milk is probably the most important source of riboflavin in the average American's diet, everything within reason should be done to preserve this all-important vitamin. Ascorbic acid is present in rather important quantities in fresh raw milk, but during processing, distribution, and storage this vitamin, too, is frequently depleted to a great extent. Many foods in the normal diet contain rather liberal quantities of vitamin C so its loss is of less importance from the nutritional point of view.

It has been demonstrated that light, particularly direct sunlight, causes a flavor defect in milk which has been variously described as the "sunlight," "activated," or "burnt" flavor (1, 2, 3, 4, 5). This defect has been a matter of considerable concern among distributors who have been confronted with numerous complaints and "returns" of milk having this off flavor. It would seem logical that milk which is subjected to light for a period of time suffi-

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cient to produce the "sunlight" flavor, would also suffer a considerable loss of riboflavin and ascorbic acid.

It is generally assumed that when the routeman leaves a bottle of milk on the customer's doorstep, the job of the distributor is done. Whether this should or should not be the case, the fact still remains that there exists a very critical period between the time the milk is placed on the doorstep and the time it is taken into the home and placed in the refrigerator. This period of exposure to light, if sufficiently long, could convert an otherwise good quart of milk into one of inferior quality.

In order to ascertain the magnitude of this problem it seemed logical that a study should first be made to determine how long housewives leave milk exposed after it is delivered. Such a study was made during the past summer and fall, together with a rather thorough laboratory investigation of "sunlight" flavor development and vitamin losses during exposure to both sunlight and shade.

METHODS

A total of 13 milk routes in the cities of Columbus, Cleveland, St. Louis, and Boston were carefully studied to determine how soon after delivery the milk was taken in by the housewife. Briefly, the procedure employed in these studies was as follows: The investigator, accompanied by a route supervisor or someone well acquainted with the route, followed the route truck and first recorded how many customers took in their milk within five minutes. Those who did not take in their milk within this period were checked again after 30 minutes and again at 60, 90 and up to 150 minutes if the milk still remained on the doorstep at time of previous re-check. On re-check visits there was naturally some overlapping of time, but this was maintained at a maximum of plus or minus five minutes, which it was felt would cancel out in the overall study. Compact routes in middle-class residential sections were chosen for these studies in order to get a good cross-section picture of the average urban community. In each study routes were selected in different parts of the city so as to get a more composite picture of the particular market.

In laboratory trials when samples of milk were exposed to sunlight or shade, a sufficient number of bottles were exposed so that one bottle could be removed from the group at the end of each of three time intervals. The bottles were placed on a cement surface in the direct sun. Samples exposed in the shade were placed on the opposite side of the building on a similar surface.

Riboflavin analyses were conducted according to the method of Hand (6) with slight variations, and with the aid of a Coleman photofluorometer for measuring fluorescence. Ascorbic acid determinations were made by the conventional direct titration method employing sodium 2,6-dichlorobenzenoneindophenol dye.

In the absence of more accurate equipment for the measurement of light intensity, a Weston light meter was used for this purpose, readings being taken by holding the instrument about one foot above the exposed surface of the bottles.

When organoleptic examinations were made for the presence of the "sunlight" flavor, the samples were tasted immediately after exposure and again after being stored for 24 hours in a household refrigerator.

RESULTS

Route Studies

The studies conducted in Columbus, Cleveland, St. Louis, and Boston revealed a very interesting cross-section picture regarding the care of milk after it is delivered to the customer. The data tabulated and presented in table 1 represent a compilation of all data taken during these studies and although they are self-explanatory, some comment should be made regarding them. At the outset, it will be noted that the per cent of deliveries taken in at the various periods appears to vary considerably among the four cities. In St. Louis, for example, 71.9 per cent of the housewives took in the milk within five minutes after delivery, while only 29.6 per cent of the deliveries were taken in within this period in Cleveland. However, when the data are examined further, it will be noted that a total of 48.9 per cent of all deliveries made in Cleveland were placed in delivery boxes or taken into the house, apartment, or refrigerator by the routeman, while only 2.6 per cent of the total were so treated in St. Louis. Columbus and Boston were similar as regards the per cent of deliveries taken in within five minutes after delivery, but Boston routemen placed considerably more milk inside of homes, apartments or in household refrigerators. Since milk placed in delivery boxes or inside homes, apartments or refrigerators is not subjected to light, it was considered "protected" in this study.

Upon examination of the data under the heading of "Total milk protected," it will be observed that all four cities were fairly uniform with respect to the total percentage of customers who actually took in their milk within five minutes or had it protected from light at time of delivery. The weighted averages show that approximately 70 per cent of all deliveries either were taken in by the housewife within five minutes or placed in the house, apartment, delivery box or refrigerator by the routeman. Between the five-minute check and the 30-minute re-check period an additional 10 per cent of deliveries were taken in, and at the 60- and 90-minute re-checks about seven and six per cent, respectively, was removed from the doorstep. During the last hour, between the 90- and 150-minute re-checks, an additional three per cent was taken in by the housewife. The number of customers who left their milk out more than 150 minutes represented 3.3 per cent of all deliveries. It is interesting to note that in all four cities this percentage was

TABLE 1

A summary of route studies in four cities to determine the length of time milk remains exposed to light after delivery

Customer	Number*				%					Total milk protected† %				
	Columbus	St. Louis	Cleveland	Boston	Columbus	St. Louis	Cleveland	Boston	Weighted average	Columbus	St. Louis	Cleveland	Boston	Weighted average
Milk taken in within 5 min.	600	334	163	142	53.9	71.9	29.6	48.8	51.3	63.2	73.8	78.5	70.8	69.6
Milk taken in within 30 min.	748	383	191	174	67.2	82.5	34.7	59.8	61.9	76.5	85.1	83.6	81.8	80.4
Milk taken in within 60 min.	661‡	419	218	202	73.6	90.3	39.6	69.4	68.6	83.9	92.9	88.5	91.4	87.7
Milk taken in within 90 min.	931	431	241	212	83.6	92.9	43.8	72.9	75.1	92.9	95.5	92.7	94.9	93.6
Milk taken in within 150 min.	971	439	263	217	87.2	94.6	47.8	74.6	78.3	96.6	97.2	96.7	96.6	96.7
Milk left in box by routeman	31	6	113	7	2.8	1.3	20.5	2.4	6.4					
Milk placed in house, apt. or refrigerator by routeman	74	6	156	57	6.6	1.3	28.4	19.6	12.0					
Milk left out more than 150 minutes	38	13	18	10	3.4	2.8	3.3	3.4	3.3					

* 2419 customers studied:
Columbus = 1114 customers on five routes of three different companies.
St. Louis = 464 customers on three routes of two different companies.
Cleveland = 550 customers on three routes of three different companies.
Boston = 291 customers on two routes of one company.

† Includes milk taken in by customers, plus that put in box, apartment or house by routeman.

‡ No data taken at 60 minutes on first Columbus study.

very uniform. As a matter of fact all 13 routes in the different cities were very similar in this respect, the extremes being 2.4 to 4.0 per cent.

It is felt that the data shown here represent a fairly accurate cross-section picture of the promptness exhibited by customers in removing their milk from the doorstep after delivery. While it is appreciated that markets vary considerably in their delivery practices, the above tabulation should represent a relatively accurate index for evaluating the importance of this problem from the standpoint of vitamin losses and off-flavor development.

The Effect of Sunlight on Milk

Vitamin losses. Investigators who have published data on the photolysis of riboflavin and ascorbic acid in milk fail to agree on how much riboflavin is lost during a definite period of exposure to sunlight. As has been pointed out by Holmes and Jones (7) some of this is probably due to the varying intensities of sunlight to which the samples are subjected as well as to variations in the temperature of the milk during exposure. Some investigators employed quart-size milk bottles while others used pint or one-half pint sizes. It would seem logical that the contents of the smaller size containers would naturally warm up faster and would also be subjected to a greater surface exposure per unit volume.

In order to determine this relationship, experiments were conducted wherein homogenized milk was filled into two-quart, one-quart and one-half pint square flint glass milk bottles and exposed to strong direct sunlight for periods up to two hours. A duplicate series was exposed simultaneously for the same interval periods on the shaded side of the building.

The results of a complete and representative experiment are recorded in tables 2 and 3. The pronounced difference in vitamin losses of the same milk exposed in different sizes of milk bottles is clearly evident. From this and several other comparisons it was demonstrated that the riboflavin losses in one-half pint bottles were roughly twice as much as those found in two-quart bottles. When the riboflavin losses shown here are compared with those reported by other workers, where the same type of milk was exposed to bright sunshine in the same size bottles and at similar temperatures, there is good agreement. Other experiments, conducted at lower outside temperatures and at lower intensities of sunlight gave results which also agree quite well with most other published work. On the basis of eight trials employing quart bottles of homogenized milk, exposed to sunlight intensities ranging from 160 to 500 Weston degrees and at temperatures ranging from 52 to 88° F., the following percentage ranges for loss of riboflavin were experienced: one-half hour, 10.2–13.9 per cent; one hour, 18.6–28.5 per cent; and two hours, 37.2–45.0 per cent. All sunlight exposures were conducted on clear days between the hours of 10 A.M. and 2 P.M.

The ascorbic acid data in table 2 as well as that obtained in numerous

TABLE 2

*The effect of bottle size on the magnitude of vitamin loss and flavor development in homogenized milk exposed to sunlight**

No.	Size of milk bottle	Hours exposed	Weston light reading	Temperature of milk after exposure	Mg./liter riboflavin	Per cent loss	Mg./liter ascorbic acid	Per cent loss	Degree of sunlight flavor	
									Immediately	24 hours
				°*F.*						
1	Control milk			52	1.37		12.7		–	–
2	2 quart	½	300	67	1.23	10.2	5.2	59.0	++	++++
3	1 quart	½	"	70	1.23	10.2	2.7	78.7	++	++++
4	½ pint	½	"	77	1.14	16.8	0.2	98.4	+++	++++
5	2 quart	1	350	74	1.07	21.9	2.3	81.9	++	++++
6	1 quart	1	"	78	0.98	28.5	0.1	99.0	++	++++
7	½ pint	1	"	86	0.79	42.3	0.0	100.0	+++	+++++
8	2 quart	2	"	83	0.86	37.2	0.9	93.0	+++	+++++
9	1 quart	2	"	87	0.76	44.5	0.0	100.0	+++	+++++
10	½ pint	2	"	97	0.42	69.3	0.0	100.0	++++	+++++

* Clear day with unrestricted sunlight. Time 10 A.M. to 12 M. Outside temperature 75–78° F.

TABLE 3

*The effect of bottle size on the magnitude of vitamin loss and flavor development in homogenized milk exposed to shade**

No.	Size of milk bottle	Hours exposed	Weston light reading	Temperature of milk after exposure	Mg./liter riboflavin	Per cent loss	Mg./liter ascorbic acid	Per cent loss	Degree of sunlight flavor	
									Immediately	24 hours
				°*F.*						
1	Control milk			52	1.37		12.7		–	–
2	2 quart	½	25	61	1.37	0.0	11.2	11.8	–	–
3	1 quart	½	"	62	1.38	0.0	10.2	19.7	–	–
4	½ pint	½	"	65	1.35	1.5	7.9	37.8	–	±
5	2 quart	1	"	65	1.31	4.3	9.1	28.3	–	±
6	1 quart	1	"	65	1.30	5.1	7.3	42.5	±	+
7	½ pint	1	"	67	1.23	10.2	5.5	56.7	+	++
8	2 quart	2	"	67	1.26	8.0	6.1	52.0	+	++
9	1 quart	2	"	68	1.21	11.7	5.2	59.0	++	+++
10	½ pint	2	"	71	1.14	16.8	2.4	81.1	++	+++

* Uniform shade. Outside temperature 75–78° F.

other trials clearly demonstrate the extreme photolability of this vitamin and further confirms the conclusion of Holmes and Jones (7) that little if any ascorbic acid remains in one-half pint bottles after exposure to sunlight for 30 minutes. As was the case with riboflavin, the losses of ascorbic acid were less in quart and two-quart bottles than in the one-half pint size.

The results obtained with shaded samples (table 3) demonstrate the great difference in the rate of vitamin destruction between milks exposed to direct sunlight and those held under shaded conditions. In this and other comparative trials the loss of riboflavin in the shade seldom exceeded 25 per cent of that for a comparable sample exposed simultaneously to the direct sunlight. On cloudy or rainy days the losses of riboflavin were usually considerably less than those exposed in the shade on bright days. Twenty trials, in which samples were held in the dark for 24 hours after exposure, confirmed data of Stamberg and Theophilus (10) which demonstrated that the destruction of riboflavin ceases immediately upon removing the samples from the light. On the other hand, ascorbic acid losses continued at a rather rapid rate after exposure.

Ascorbic acid losses in the shade were surprisingly high and only further confirm the extreme sensitivity of this vitamin to light.

Flavor. The development of the so-called "sunlight" flavor was given considerable attention in the course of these studies. Because of the relatively high degree of oxidative stability displayed by most milk produced in summer and fall, very few of the samples exposed to light developed the "oxidized" flavor. However, the development of the characteristic "sunlight" flavor was very pronounced and appeared very quickly. The flavor data shown in tables 2 and 3 are recorded in terms of plus signs, depending upon the intensity of the flavor. These data representing the opinions of three judges, show that the flavor develops rapidly and that it increases in intensity during storage for 24 hours. In some trials the initial intensity of the flavor immediately after exposure or the rate of its development during subsequent storage was much less than in others, but with all freshly pasteurized milks the defect was very evident immediately after exposure.

The development of the sunlight flavor in samples held in the shade is somewhat surprising since the light intensity was relatively low. With one-half pint samples the flavor was frequently evident after one-half hour in the shade. However, with quart bottles of milk, little or no "sunlight" flavor developed until after more than one-half hour's exposure.

Route and Home Care of Milk

In order to have a more complete picture of the problems of vitamin loss and "sunlight" flavor development under actual conditions of milk distribution, a series of studies was conducted.

From the time the milk leaves the milk plant until the routeman has the load delivered, some six hours frequently elapse. With conventional covered trucks and solid wall (wood) milk cases little direct sunlight actually reaches the bottle surfaces. Wire cases, on the other hand, subject the milk to considerable daylight especially near the front part of the truck. Numerous trials were conducted wherein random quarts of milk were removed from the truck before leaving the plant and every hour during the period of delivery. Some trucks carried both wood and wire cases for the purpose of the experiment. The samples were placed in a dark box and taken to the laboratory for analysis.

The data taken in this study are recorded in table 4. As might be expected, the vitamin losses were somewhat higher in bottles of milk carried in wire cases than in those carried in wooden cases. The riboflavin losses were negligible except where milk was on a route for five hours or more. It should also be pointed out that trial 1 was conducted on a very hot, bright summer's day when higher than normal losses could be expected. This particular trial gave the highest percentage losses experienced.

TABLE 4

The loss of riboflavin and ascorbic acid in route delivery trucks

Trial No.	Weather conditions	Type of milk case	Time on route	Riboflavin loss	Ascorbic acid loss	Degree of sunlight flavor
			hr.	*per cent*	*per cent*	
1	Clear Bright Hot	Wood	1	0.6	26.8	–
		Wire	1	1.2	27.5	–
	"	Wood	2	4.3	26.1	–
		Wire	2	4.6	34.1	–
	"	Wood	5½	9.1	63.0	–
		Wire	5½	17.7	90.6	–
2	Cloudy Warm	Wood	1	0	0	–
		Wire	1	0	5.8	–
	"	Wood	3	0	1.9	–
		Wire	3	0.6	11.7	–
	"	Wood	5	1.8	9.8	–
		Wire	5	6.2	40.0	–

Losses of ascorbic acid again were quite high yet not surprising, in view of the photolability of this vitamin. Although vitamin losses are low in milk carried on retail trucks, there is a potential danger if routemen are careless, especially in exposing wire cases of milk to the direct sunlight for long periods of time.

Since some housewives frequently allow milk to be exposed to light in the kitchen, it was of interest in this connection to determine how such treatment affected riboflavin, ascorbic acid, and the flavor of milk. Two trials were conducted in which quarts of homogenized milk were allowed to stand for two hours on a table in a bright kitchen having an east exposure.

Samples stood for two hours during the forenoon, but were not in direct sunlight. In these two trials riboflavin losses of 3.2 and 4.0 per cent and ascorbic acid losses of 52.0 and 67.4, respectively, were found. In both trials the milk developed a slight but definitely discernible "sunlight" flavor.

DISCUSSION

It is clearly evident that the exposure of milk to sunlight can cause important losses of riboflavin and ascorbic acid as well as impair the flavor of the product. The potential danger periods are those during which the milk is on the truck and after the product is on the customer's doorstep.

Ascorbic acid losses, while large, are not of critical importance because of the liberal quantities of this vitamin acquired from other foods in the average diet. The loss of riboflavin, however, is important because milk constitutes our most important source of this vitamin.

While the losses of riboflavin reported here and by others previously, do appear significant, it is the opinion of the writers that the problem is far less critical than has been assumed by previous workers. From the results of the route studies reported here it is evident that nearly 70 per cent of all milk deliveries are removed from the doorstep within five minutes, and therefore, lose little or no riboflavin.

If we assume that all deliveries in this study were equal as to the number of quarts of milk per customer and that the riboflavin losses during exposure to sunlight were 12 per cent for one-half hour, 20 per cent for one hour, 30 per cent for one and one-half hours, 40 per cent for two and one-half hours, and 60 per cent for that milk left out for more than two and one-half hours, we can calculate, approximately, the percentage of riboflavin that is lost. In so doing we must also consider the facts that, 1) the sun shines only about 55 per cent of the time (average of U. S. Weather Bureau records for areas studied), and 2) at least one-half of all milk that is left out is on the shady side of the street or in other shaded places. Since losses under shaded conditions do not exceed 25 per cent of that experienced in direct sunlight, this factor must be considered in any calculation. On the basis of the above facts and assumptions it has been calculated that about 3.25 per cent of the riboflavin is lost after milk is delivered to the customer's doorstep. This figure is, undoubtedly, too high because of the fact that, in general, milk is delivered in the morning when the light intensity is lower than during the hours when laboratory trials were run. Support for the view was found in analyses of samples picked up during the route studies. Three quarts of milk (each from different days) which were known to have been exposed to the direct morning sun for two and one-half hours, showed riboflavin losses of 26.2, 28.6 and 33.1 per cent in contrast to the 40.0 per cent average assumed above. Another intangible factor, which is not considered in the above calculation, is the question of the temperature during the period of exposure, which is known to affect the rate of riboflavin loss. During winter months, when milk is exposed at lower temperatures, losses would be expected to be less. Furthermore, the route studies reported here were conducted in typical residential sections of cities and it might be expected that the percentage of apartment deliveries ("protected milk") is higher, for the nation as a whole, than is shown here.

It is the feeling of the writers that the problem of the "sunlight" flavor is of greater significance than the loss of part of the riboflavin. Most milk that is subjected to sunlight for as little as one-half hour develops the very unpleasant "sunlight" flavor.

It is appreciated that the small average loss of riboflavin demonstrated here is somewhat misleading when it is considered that in many cases, where housewives are careless in the matter of caring for milk, the resulting loss might be an important factor in the nutrition of the families in question. However, it is felt that the correction of this situation will not require any revolutionary changes in milk containers or in conventional delivery practices, but can be largely rectified by a program of consumer education. Such a program initiated through the combined efforts of distributors and dairy promotional agencies can do much to disseminate such information to consumers and thereby make milk a more nutritious and more palatable food.

SUMMARY

Approximately 70 per cent of all retail milk deliveries are removed from the doorstep or protected from light within five minutes after delivery while 3.3 per cent of customers leave milk exposed for more than two and one-half hours.

Under experimental conditions of exposing milk to sunlight, the loss of ascorbic acid is extremely rapid with only insignificant quantities remaining after 30 minutes. Losses of this vitamin in the shade are also quite rapid.

Riboflavin losses vary depending upon the intensity of sunlight, the temperature of the milk, and the size of bottles in which the milk is exposed. Under conditions of direct sunlight exposure for extended periods of time, milk loses significant quantities of this vitamin. On the other hand, the loss of riboflavin from milk exposed to shade does not exceed 25 per cent of that experienced in the direct sunlight.

The photolysis of riboflavin stops when milk is removed from light while losses of ascorbic acid continue at a somewhat slower rate.

The "sunlight" flavor, which develops in milk during exposure, appears to be a more critical problem than the loss of some of the riboflavin. On the basis of route studies and certain basic assumptions, it is believed that the average loss of riboflavin in all milk delivered does not exceed 3.25 per cent.

It is the feeling of the writers that a program of consumer education in the care of milk can be of great value in maintaining the palatability and nutritional qualities of this important food.

Further practical and fundamental studies are being conducted on the matters of "sunlight" flavor development and vitamin losses, the results of which will be reported in the near future.

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THE EFFECT OF SULFANILAMIDE UPON THE LIVABILITY AND METABOLISM OF BOVINE SPERMATOZOA

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The importance of bacteriological control of semen produced for use in artificial breeding of dairy cattle has been presented in reports by Gunsalus *et al.* (3, 4). These workers, while able to keep bacteria of semen at a minimum by aseptic methods of collection and handling, were never able to eliminate bacterial contamination entirely. Some bacterial types were normal inhabitants of the male genital tract, others were invaders of the tract, and still others were introduced into the semen at collection or later. *Pseudomonas aeruginosa* organisms, apparently harbored deep in the reproductive tract of some bulls, were shown to be deleterious to conception in artificial breeding. No one has yet been able to determine the effect of bacteria on the results of metabolic studies of spermatozoan physiology, though most workers have dismissed the problem as being of little or no consequence. Finally, the possible transmission of certain infections to cows through use of semen containing the responsible bacterium has not been thoroughly investigated.

These facts suggested the desirability of studying the effects of certain bacteriocidal or bacteriostatic agents on the livability and metabolism of bull spermatozoa. Shettles (10) in 1940 reported that survival and activity of human spermatozoa were not adversely affected by the addition of up to 160 mg. of sulfanilamide, or of sulfapyridine per 100 ml. of Baker's fluid used for diluting human semen. Thus, it was logical that the present investigation proceed with the study of one of these chemotherapeutic compounds and its effect on bull spermatozoa. The present report deals with the effect of sulfanilamide.

EXPERIMENTAL

The problems which required solution in these investigations were as follows:

1. What levels of sulfanilamide would be required to prevent growth of the bacterial types found in semen routinely collected from bulls;
2. What effect would sulfanilamide have on the livability and metabolic activity of spermatozoa and, finally;
3. What effect would the required levels of sulfanilamide have on the semen as treated when used for routine artificial insemination? This latter

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problem is being investigated in a separate experiment and will be reported later.

Effect of sulfanilamide on bacterial growth. The first experiments were conducted using turbidimetric methods (6) for determining bacterial growth. Fresh bull semen was diluted at the rate of 1 part of semen to 10 parts of an isotonic sodium citrate solution. This solution was 3.6 grams $Na_3C_6H_5O_7.2H_2O$ per 100 ml. of water distilled over glass. To certain batches of citrate solution sulfanilamide was added at varying concentrations and all solutions were simultaneously autoclaved for 20 minutes at 15 lb. pressure.

The technique for determining the effect of various levels of sulfanilamide on bacterial growth consisted of measuring the change in density of each diluted semen sample during incubation for 72 hours at 37.5° C. The original density was due not only to spermatozoa, but to materials in the seminal plasma and bacteria. The change in nephelometer readings, made at 24-hour intervals, was considered as due to bacterial growth. Motility observations were made on each incubated sample to determine the effect of sulfanilamide on the livability of the spermatozoa.

In the first experiment 4 ejaculates were used to each of which sulfanilamide was added at levels of 0, 10, 20, 50, 100, and 200 mg. per 100 ml. In this experiment all spermatozoa with sulfanilamide added lived better than did the spermatozoa in samples containing no sulfanilamide. However, as was to be expected, bacterial growth was enormous in the samples containing no sulfanilamide, the optical density doubling in 72 hours. As bacteria made up a relatively small portion of the oriignal density, this indicates that bacterial numbers increased manyfold. However, at the 200 mg. per 100 ml. level there was only a 3.6 per cent increase in density.

In two subsequent experiments using 5 and 8 separate ejaculates and sulfanilamide levels of 0, 10, 20, 50, 100, 200, 500, and 1000 mg. per 100 ml. no increase in density was observed for the levels of 200 mg. per 100 ml. or above. This fact established 200 mg. per 100 ml. as the minimum level consistent with bacteriological control. Also, in these two experiments all levels of sulfanilamide aided in maintenance of motility as compared to the controls. The maximum increase in livability was with the 200 mg. per 100 ml. concentration, the duration of motility being depressed slightly by the 500 and 1000 mg. per 100 ml. amounts.

Level of sulfanilamide for optimum livability of spermatozoa in yolk-citrate. To determine the effect of sulfanilamide on spermatozoa under conditions comparable to those in routine artificial insemination it was necessary that the semen be diluted with the yolk-citrate commonly used here. The primary object was to determine the effect of the different amounts of sulfanilamide on spermatozoan activity. Because of the presence of the yolk no studies of the change in density could be made. However, routine microscopic examinations afforded a ready means of determining within limits the effect of sulfanilamide on bacterial growth.

For these studies fresh bull semen was diluted with the yolk-citrate diluent composed of one part of fresh egg yolk to one part of the isotonic sodium citrate solution, previously mentioned. The diluted semen was gradually cooled to the temperature of storage, 5° C., and stored in stoppered test tubes. The test tubes were opened at two-day intervals to subsample for microscopic examination of spermatozoan activity. The aliquots were obtained by use of sterile pipettes.

A series of preliminary experiments indicated that every level of sulfanilamide improved the livability of the spermatozoa, as had been the case when the yolk was not included. Additions of 1000 mg. per 100 ml., however, depressed livability as compared with the certain lower levels of added sulfanilamide. These experiments established the desired levels at from 100 to 500 mg. per 100 ml., which were used for the next experiment.

Fourteen separate ejaculates were diluted at 1:9 and 1:49 with the yolk-citrate and stored for 20 days at 5° C. These two dilution rates were chosen as the range of dilution then employed by the New York Artificial Breeders' Cooperative, Inc., for routine artificial breeding. Also, it was desired to determine if sulfanilamide additions would eliminate the dilution effect on livability previously reported from this laboratory (7).

The 14 ejaculates had a mean concentration of 967 thousand spermatozoa per mm.^{3}, mean methylene blue reduction time of 7.0 minutes, and a mean motility of 69 per cent motile spermatozoa moving at a rate of 3.3, where 4.0 is considered optimum. The samples were observed every 2 days for percentage and rate of motile spermatozoa.

The motility data of the experiment after 10 and 20 days' storage at 5° C. are shown in table 1. The observations made at each 2-day interval were used for a statistical analysis of variance. Highly significant differences were shown for sulfanilamide levels with 300 mg. per 100 ml. being optimum. In addition, the statistical interactions of ejaculate × treatment, and dilution × treatment were highly significant, aso. Sulfanilamide had no apparent influence in preventing the dilution effect, for the difference between dilution rates was highly significant statistically. The mean motility of the entire experiment for the 1:9 rate was 43 per cent, and for 1:49, 37 per cent.

While objective bacterial counts were not made on these diluted semen samples, microscopic observations at the time of motility examinations indicated great bacterial growth after 10 to 12 days of storage in those samples without added sulfanilamide. On the other hand, no bacteria were observed after storage at the 200 mg. per 100 ml. or higher concentrations of sulfanilamide.

Effect of sulfanilamide on glucose utilization and lactic acid production.

TABLE 1

The effect of sulfanilamide additions to yolk-citrate upon spermatozoan livability. Mean of 14 ejaculates

Days of storage	1:9*						1:49*					
	mg.% sulfanilamide						mg.% sulfanilamide					
	0	100	200	300	400	500	0	100	200	300	400	500
	% motile spermatozoa						% motile spermatozoa					
10	35	44	54	58	46	40	24	39	46	48	40	34
20	7	16	20	26	16	9	0	6	10	15	8	1
	Rate of motility						Rate of motility					
10	1.1	1.3	1.6	1.6	1.4	1.2	0.8	1.1	1.3	1.3	1.1	1.0
20	0.5	0.8	0.9	0.9	0.8	0.5	0.0	0.5	0.5	0.7	0.5	0.4

* Parts of semen to yolk-citrate.

Earlier reports from this laboratory (8, 13) indicated that glucose loss in semen samples diluted with yolk-citrate and stored for 10 days at 5° C. was not related to the livability of the spermatozoa. However, lactic acid accumulation in such samples was highly correlated with livability.

Later, Salisbury and VanDemark (9), in studying the dilution effect. found that semen ejaculates when diluted to a greater extent with yolk-citrate produced somewhat more lactic acid per 10^8 cells than did the same semen which was diluted less. On the other hand, the proportion of the glucose which disappeared on storage, and which was recovered as lactic acid, was much lower for the more dilute samples. It was felt that this unrecovered glucose might have been oxidized at a greater rate by the less concentrated spermatozoa or that bacteria might have oxidized it.

Thus, in the present investigation it was of interest to know not only how sulfanilamide might effect glycolysis of spermatozoa, but what effect it would have on the recovery of glucose as lactic acid. Therefore, 10 ejaculates of the semen diluted with yolk-citrate and stored for 6, 12, and 18 days were analyzed for glucose and lactic acid. Glucose analyses were

TABLE 2

The effect of sulfanilamide upon glucose and lactic acid metabolism of ejaculated bull semen stored at 5° C. in yolk-citrate for 18 days. Mean of 10 determinations

	Rate of dilution	mg.% sulfanilamide					
		0	100	200	300	400	500
mg.% glucose loss	1:9	105	86	80	66	63	60
	1:49	82	46	19	10	8	5
mg.% lactic acid gain	1:9	82	83	89	96	92	76
	1:49	24	27	26	22	20	20
% glucose utilized recovered as lactic acid	1:9	78	97	111	145	146	127
	1:49	29	59	137	220	250	400

determined by the method of Horvath and Knehr (5) and lactic acid by the method of Barker and Summerson (1). Determinations were made on Somogyi filtrates (11).

The data on glycolysis during 5° C. storage for 18 days are shown in table 2. The breakdown of glucose to lactic acid, glycolysis, was actually stimulated by sulfanilamide in some concentrations. At the 1:9 dilution maximum stimulation was at the 300 mg. per 100 ml. concentration. For the higher dilution rate maximum lactic acid production was obtained with the 100 and 200 mg. additions.

What appears to be of equal interest in table 2 is the data concerning the percentage of glucose loss recovered as lactic acid. In those tubes without sulfanilamide the percentage of glucose loss recovered as lactic acid was relatively small, but increased with each addition of sulfanilamide. Thus it is possible that bacteria utilized a part of the glucose not recovered as lactic acid in this and the previous investigations. However, as will be shown in the next section, sulfanilamide depresses oxygen consumption of spermatozoa and may function not only to suppress bacterial growth, but to suppress oxidative systems in diluted semen, including the possible oxidation of lactic acid or oxidation of glucose by the spermatozoa themselves.

The recovery of lactic acid in excess of glucose utilized probably is due to the metabolism of glycogen or other carbohydrate materials which the authors have found to be present in both semen and egg yolk.

Effect of sulfanilamide on oxygen consumption by washed spermatozoa. For this study 10 fresh bull-semen ejacualtes were used. The semen was cooled and then centrifuged. The seminal plasma was removed by aspiration and the spermatozoa were washed twice with 0.9 per cent NaCl, which was added at the same temperature as the spermatozoa. The washed spermatozoa were then suspended in Krebs calcium-free Ringer phosphate fluid (12). The concentration of spermatozoa was determined turbidimetrically and the sample diluted to a final concentration of 1 billion spermatozoa per ml. Three-quarters of a ml. of this spermatozoan preparation was placed in the Krebs calcium-free fluid in the Warburg flasks. The final concentrations of 0, 100, 200, 300, and 500 mg. per 100 ml. of sulfanilamide in the flasks was obtained by using Krebs fluids with appropriate sulfanilamide concentrations. The final concentration of spermatozoa was 250 million per ml.

The oxygen consumption was measured by the direct method of Warburg under air at 37.5° C. (2). The CO_2 was absorbed by 0.2 ml. of 20 per cent KOH in the center cup containing a small piece of filter paper. The manometers were shaken at the rate of 120 oscillations per minute. After one-half hour of endogenous oxygen consumption 0.25 ml. of a 1.2 per cent glucose solution was tipped from the sidearm into the reacting flask.

The data are presented in table 3. They show a marked effect of increasing sulfanilamide concentration on the utilization of oxygen by washed spermatozoa. The effect was more marked in the absence of glucose than in its presence, for glucose itself apparently reduced oxygen consumption.

TABLE 3

The effect of sulfanilamide upon the oxygen utilization of washed spermatozoa. Mean of 10 ejaculates*

	mg.% sulfanilamide				
	0	50	100	300	500
Endogenous for ½ hour	12.3	10.5	9.4	7.1	6.3
100 mg.% glucose for 1 hour	5.7	5.5	5.5	4.7	4.2

* Expressed as mm.^{3} oxygen utilized/10^8 spermatozoa/hour (ZO_2).

DISCUSSION

The experiments presented in this paper demonstrate that sulfanilamide may be added to ejaculated bull semen diluted with yolk-citrate without decreasing the livability of the spermatozoa. A level of 300 mg. of sulfanilamide per 100 ml. of diluter gave, on an analysis of variance, a highly significant improvement in spermatozoan livability, it prevented bacterial growth, and appears to be optimal on the basis of these experiments.

Sulfanilamide markedly affected the glucose and lactic acid metabolism of diluted bull semen. The addition of 300 mg. of sulfanilamide per 100 ml. of diluter gave an increased accumulation of lactic acid at the 1:9 dilution whereas the greatest accumulation was at the 100 mg. level with the 1:49 dilution. Glucose utilization was greatest in the conrols; sulfanilamide at all levels, depressed the metabolism of this substance. Also, sulfanilamide depressed the oxygen utilization by washed spermatozoa under basal conditions, as well as in the presence of added glucose.

For certain of these experiments 3 different batches of sulfanilamide have been simultaneously tested. The results were similar for all batches. In the routine handling of the citrate buffer with added sulfanilamide precautions should be taken to keep it away from the direct rays of the sun.

The effect of sulfanilamide on fertility of diluted bull semen is under investigation. Preliminary data indicate that it has no deleterious influence on conception rate. However, at the present writing definite conclusions on this problem cannot be drawn.

A method of collection of bull semen free from bacteria is not now available. However, it would appear from these studies that in sulfanilamide or other sulfonamides a key to the control of bacteria in semen and control of possible spread of certain infections by artificial breeding is available. The action of sulfanilamide is selective for it is either bacteriocidal or bacteriostatic, but permits bull spermatozoa to live longer.

SUMMARY

1. The addition of 300 mg. of sulfanilamide per 100 ml. of yolk-citrate diluent, gave a significant improvement in the livability of ejculated bull spermatozoa over a 20-days-storage period and prevented bacterial growth.

2. Sulfanilamide depressed glucose and oxygen utilization at all concentrations studied.

3. The accumulation of lactic acid was increased both absolutely and in relation to the glucose utilized in the presence of sulfanilamide.

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VITAMIN C, HYDROGEN PEROXIDE, COPPER AND THE TALLOWY FLAVOR IN MILK

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In a preceding paper it has been disclosed that the reaction which produces the tallowy flavor can be inhibited by quick and complete oxidation of ascorbic acid in milk and that the reaction can be induced again by the addition of ascorbic acid (5).

This phenomenon together with the results obtained to date tends to indicate that the reaction responsible for the breakdown of the lipid fraction of the milk is a coupled reaction, possibly initiated under favorable conditions by a catalyst. In this connection it is of importance to note that ascorbic acid is the only form of vitamin C present in milk at the time of its removal from the mammary gland, or immediately after pasteurization (4), whereas the tallowy flavor is ordinarily detected in milk containing considerable amounts of dehydroascorbic acid as well. Equally important is the stimulative effect of the partial photochemical or chemical oxidation of the ascorbic acid in the milk to dehydroascorbic acid upon the development of the tallowy flavor during its subsequent storage in the dark (5). It suggests that the oxidation of the lipids of the milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic and dehydroascorbic acids has been established. Accordingly, copper as an accelerator of ascorbic acid oxidation by atmospheric oxygen to dehydroascorbic acid might help to bring the system more quickly to its critical point, or, in other words, to a condition under which the reaction is permitted either to deviate from its original course or to be replaced by a new one.

Furthermore, there is a good reason to believe that the promoter (an enzyme) of ascorbic acid oxidation with added H_2O_2 is largely responsible for its quick conversion in the milk to dehydroascorbic acid, and that if H_2O_2 were formed in the photochemical reaction (3) it might oxidize the residual ascorbic acid, as in the case of milk to which it has been added. Consequently, it was assumed that if a trace of H_2O_2 were present in the milk at the point when all of the ascorbic acid was oxidized, it might initiate in the presence of a suitable catalyst the breakdown of the lipid fraction of the milk (5). As a matter of fact, the tallowy flavor was sometimes induced by copper, added to milk at the end of the first reaction, even though less effectively as compared with that in milk containing ascorbic acid. Thus, the reason for the depletion of milk of its total vitamin C content prior to storage was to effect the destruction of the products of the first reaction by the following heat treatment that takes place in pasteurization. In such

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a milk the tallowy flavor was not induced in the presence of copper (0.1 p.p.m.).

It was apparent therefore that at the end of the first reaction the residual H_2O_2, together with the catalyst, could constitute an additional factor for the promotion of the tallowy flavor in milk.

The following experiment was carried on to test further the theory that the reaction which produces the tallowy flavor is initiated more rapidly when a certain pressure between ascorbic and dehydroascorbic acids has been established. A sample of fresh mixed milk was pasteurized at 61.6° C. (143° F.) for half an hour and then quickly cooled to room temperature, at which point the ascorbic acid content of the milk was increased to 50 mg. per liter. Part of the milk was then treated with a carefully calculated quantity of 30 per cent H_2O_2 solution to produce milk containing dehydroascorbic acid only. Immediately after, by dilution of the portion of milk containing 49.43 mg. of ascorbic acid with the one containing 45.69 mg. of dehydroascorbic acid per liter, respectively, a series of samples were prepared which had progressively decreasing ascorbic acid contents but retaining their total vitamin C content at approximately the same level. The samples were stored at 0 to 5° C. (32° to 41° F.) in the absence of light. The ascorbic acid content of the milk was followed by direct titration with 2,6-dichlorophenol-indophenol in acid solution, but the total vitamin C content was determined by the Gunsalus and Hand method (2).

The data presented in figure 1 are rather conclusive in showing that there is a relationship between the pressures of ascorbic and dehydroascorbic acids on one hand and the production of the tallowy flavor on the other; and that the breakdown of the lipid fraction of the milk is initiated more readily when ascorbic and dehydroascorbic ratio is less than one or approaching one. Since dehydroascorbic acid in aqueous solutions is less stable than the reduced form of vitamin C, it would appear that unless the rate of ascorbic acid oxidation to dehydroascorbic acid surpasses that of dehydroascorbic acid to non-reducible substances, the necessary amount of dehydroascorbic acid could not be accumulated in the milk.

Accordingly the protective influence of ascorbic acid when added in large quantities to milk or to any other fat-containing food product can be attributed to the exhaustion of dissolved oxygen prior to the establishment of a favorable equilibrium between these two forms of vitamin C. If atmospheric oxygen is not made available at the point of the depletion of the occluded oxygen, the chances are that dehydroascorbic acid would be decomposed before the arrival of the new supply of oxygen. As a consequence, the oxidative process must start over again. It does not imply, however, that, because of the limited supply of oxygen made available at each successive point, the oxidative deterioration of the fat could be postponed indefinitely. It is necessary to consider as well the effect of the time of storage upon the susceptibility of fat to oxidation (induction period), and that with the progress of time the conditions favoring the reaction would be correspondingly augmented.

Thus the differences between the samples of milk fortified with large amounts of ascorbic acid, in their abilities to resist the reaction which produces the tallowy flavor, can be attributed to the ability of milk to promote

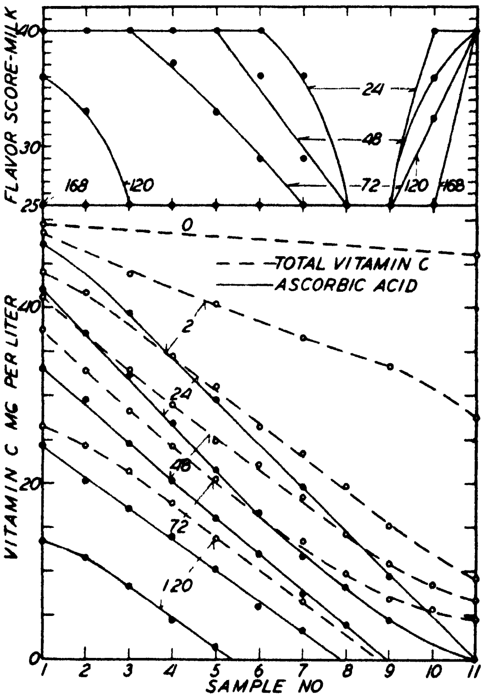

FIG. 1. The relationship between ascorbic and dehydroascorbic acids pressures in the milk (samples 1 to 11) at the end of 2, 24, 48, 72 and 120 hours holding period at 0° to 5° C. and the development of the tallowy flavor. The flavor score (the upper part of the figure) indicates: 40, no criticism; 35–40, acceptable; and 25, unsuitable for consumption.

ascorbic acid oxidation in the presence of atmospheric oxygen and the continued availability of the latter. This statement is further supported by our observations which indicated that under normal conditions the rate of ascorbic acid oxidation in so-called "susceptible milk" is apparently greater than that of less susceptible or "non-susceptible milks."

The data of figure 1 also show that with the passage of time irrespective of the initial ascorbic and dehydroascorbic acids ratios, the oxidized form of vitamin C tends to approach the level parallel to that for the ascorbic acid content of the samples.

It has already been remarked that a catalyst (an enzyme) might be responsible for the quick oxidation of ascorbic acid in milk by added H_2O_2. The evidence for this belief was the following: In a previous paper it has has been shown that, depending upon the ascorbic acid content of the fresh milk, from 0.021 to 0.03 ml. of 30 per cent H_2O_2 were needed to oxidize ascorbic acid quickly and completely. At that time it was observed that with further increase in the volume of H_2O_2 added to milk the rate of ascorbic acid oxidation was noticeably retarded. The results of this experiment, since repeated several times, are graphically presented in figure 2.

In this case the samples were prepared by dilution in sequence of milk containing 0.816 ml. of added H_2O_2 with normal milk, to produce a series of samples containing 0.816, 0.408, 0.204, 0.102, 0.051, 0.0382, 0.0286, 0.0215, 0.016, 0.012, 0.009, 0.0068 ml. of 30 per cent H_2O_2 per liter of milk, respectively.

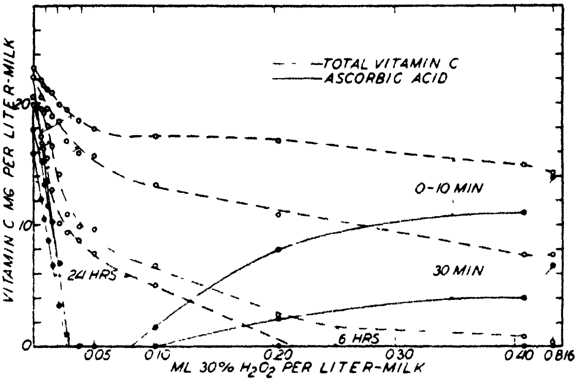

FIG. 2. The inverse relationship between the volumes of 30 per cent H_2O_2 solution added to milk and the time required to oxidize ascorbic acid either partially or completely.

The data show that the rate of ascorbic acid oxidation varied inversely with the volume of hydrogen peroxide added to milk. In the presence of 0.068 to approximately 0.08 ml. of the agent, ascorbic acid was oxidized either partially or completely within 0 to 10 minutes of time, respectively, whereas more than 30 minutes were required to complete its oxidation in the samples treated with 0.204 to 0.816 ml. of H_2O_2. This progressive retardation of ascorbic acid oxidation suggests that the reaction may be catalyzed by an enzyme, which in turn is slowly inactivated by H_2O_2. The data show also that, in the presence of as much as 0.1 ml. of H_2O_2, the oxidation of ascorbic acid to dehydroascorbic acid was promoted at a considerably faster rate than that of dehydroascorbic acid to non-reducible substances. Further increase in the volume of H_2O_2 added to milk resulted in the progressive retardation of ascorbic acid oxidation and more rapid destruction of dehydroascorbic acid. The break in the curve for the latter apparently occurred at the point coresponding to 0.2 ml. of H_2O_2; but at 0.816 ml.,

TABLE 1

The relationship between the amounts of 30 per cent hydrogen peroxide added to milk prior to pasteurization, vitamin C content, added copper, added ascorbic acid (Table 1–A) and the development of the tallowy flavor in milk subsequently held at 0 to 5° C.

Held at 0 to 5° C.	Untreated control sample		Ml. of 30% hydrogen peroxide per liter of milk prior to pasteurization at 143° F. for 30 minutes																							
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After H_2O_2 added	18.1	18.8	14.2	18.1	12.0	17.3	9.6	16.6	7.1	16.3	4.3	14.9	0.0	13.8	0.0	13.8	0.0	12.7	1.7	12.6	6.0	12.4	9.6	12.2	12.0	12.0
and pasteurized	12.0	12.7	8.1	8.5	7.1	7.8	5.3	5.7	2.8	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	8.9	12.0	5.7	7.9	4.9	6.3	3.9	4.9	0.7	2.1																
4	0.0	5.9	0.0	2.9	0.0	2.1	0.0	1.6	0.0	0.0																
	Flavor score																									
1	40		40		40		40		40		40		40		40		40		40?		40		40		40	
2	40?		40?		40?		34–T.		40		40		40		40		40		40?		40		40		40	
4	25–T.		25–T.		25–T.		25–T.		40		40		40		40		40		40		40		40		40	
8									40		43*		43		40		40		40		40		40		40	
	Copper added 0.1 p.p.m.																									
1	4.2	10.9	2.1	6.3	1.4	5.0	0.3	5.0	0.0	1.4																
4	0.0	2.7	0.0	1.6	0.0	1.0	0.0	1.0	0.0	0.0																
	Flavor score																									
1	25–T.		25–T.		25–T.		25–T.		40?		40		40		40		40		40?		40		40		40	
2									40		40		40		40		40		40		40		40		40	
4									40		40		40		40		40		36–T.?		40		40		40	
8									40		40*		40		40		36–T.		36–T.?		40		40		40	

dehydroascorbic acid was destroyed as fast as formed. The last sample, as the data in table 1–A indicate, was an exceptional one in many respects. The addition of ascorbic acid either alone or with copper to milk depleted of its total vitamin C content not only failed to induce the tallowy flavor, but also the sample lost its vitamin C content at a much faster rate than the nearest one in the same series.

The differences between ascorbic and dehydroascorbic acids in their rates of oxidation with H_2O_2 indicate, therefore, that the second reaction, involving dehydroascorbic acid oxidation with H_2O_2 to non-reducible substances, was not effected by the promoter of ascorbic acid oxidation.

These results are supported by the observations of Steinman and Dawson (12) who concluded that the rate of dehydroascorbic acid decomposition is increased by the addition of H_2O_2, apparently because of the bimolecular reaction between dehydroascorbic acid and H_2O_2. They also stated that the initial stage of decomposition is pseudomolecular in character and is not due to an oxidation which is catalyzed by a metallic ion.

Since, at the end of the first reaction, H_2O_2 can be a variable component of the new system in which the catalyst plays a part, it was of interest to learn if there is a condition under which the breakdown of the lipid fraction of the milk could proceed rapidly and to completion. Consequently, there were two problems to solve. First, to determine the effect of H_2O_2 when added to milk in excess of that required to oxidize its ascorbic acid content prior to the heat treatment upon the susceptibility of milk to tallowy flavor in the presence of subsequently added copper. Second, the ability of copper to promote the breakdown of the lipid fraction of the milk by the residual H_2O_2. For these reasons milk was treated with different amounts of 30 per cent H_2O_2 both prior to and after the heat treatment. It was thought that dehydroascorbic acid and the residual H_2O_2 would be destroyed in one case and retained in the other, at least for a period of time. Thus, two series of samples were prepared by following the same dilution technic as previously described, with the exception that the experiments were performed by using two batches of milk from the same cows on two different days.

The data presented in table 1 show that, with the exception of one or possibly two samples of milk, the tallowy flavor was not promoted by copper in the milk completely depleted of its total vitamin C content by the procedures just described. Even then the flavor developed at a comparatively slow rate, its intensity varied from day to day, and in some cases it was hard to ascertain if the flavor was present at all. It is possible that in this particular case the reaction was started during ascorbic acid oxidation with H_2O_2, which coincidently was appreciably retarded at this point (fig. 1), and, although it was not allowed to continue because the necessary components of the system were destroyed by the following heat treatment, the lipid sensitivity to copper-induced oxidation was somewhat affected.

TABLE 1–A

Ascorbic acid readded after hydrogen peroxide treatment and pasteurization

Held at 0 to 5° C.	Untreated control sample		Ml. of 30% hydrogen peroxide per liter of milk prior to pasteurization at 143° F. for 30 minutes																					
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After pasteurized	12.0	12.7	8.1	8.5	7.1	7.8	5.3	5.7	2.8	3.2	0.0	0.0	0.0	0.0	0.0		0.0		0.0		0.0		0.0	..	0.0	
and A.A. added	32.6	33.3	29.0	29.0	27.6	28.7	26.6	26.9	23.0	23.7	20.9	21.2	20.2	19.8	20.2	19.1	19.8	20.2	20.9	19.5	20.2	19.1	20.9	19.8	20.9	19.5
10 min.	32.6	33.3	27.9	29.0	27.6	28.3	26.2	25.8	22.3	24.0	20.2	20.9	19.5	20.2	19.1	19.8	19.5	19.8	18.4	19.5	17.7	18.4	14.5	17.0	10.6	11.0
1	27.9	32.2	24.8	29.0	24.0	27.9	21.9	25.8	19.8	22.6	16.6	19.8	15.9	19.1	15.9	18.4	15.9	18.4	15.6	19.1	11.3	16.3	2.5	9.6	0.0	1.4
4	14.9	24.5	12.3	20.3	10.7	19.2	8.5	17.3	6.7	14.4	4.3	11.7	3.2	10.7	1.6	9.0	1.0	9.0	3.7	10.4	1.9	7.2	0.0	2.1	0.0	0.0
	Flavor score																									
1	40		40		40		40		40		40		40		40		40		25–T.		25–T.		30–T.		40–Ca.	
2	40		40		40		40		40		40		40*		40		25–T.								40	
4	25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.										40	
	Copper added 0.1 p.p.m.																									
130 min.	31.2	32.6	26.6	27.9	26.2	27.6	23.7	25.8	20.9	22.3	18.0	20.2	17.7	19.1	17.7	18.4	17.7	18.8	17.2	18.4	13.8	17.0	6.0	13.1	1.8	4.2
1	20.2	28.7	17.7	24.8	17.0	23.7	16.6	22.7	13.1	19.8	10.3	17.2	9.2	16.3	7.8	15.6	6.7	15.6	6.0	14.9	1.4	11.3	0.0	4.6	0.0	0.0
4	0.0	11.2	0.0	9.1	0.0	8.5	0.0	6.9	0.0	6.4	0.0	5.9	0.0	5.0	0.0	4.3	0.0	4.5	0.0	4.5	0.0	3.2		1.3		
	Flavor score																									
1	40		40		40*		39–T.		40		40		25–T.		25–T.		25–T.		25–T.		25–T.		39–T.		40–Ca.	
2	25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.										36–T.		40	
4	.																						25–T.		40	

A.A. = Ascorbic acid; T.C. = Total vitamin C.

Remarks: Flavor score indicates: 40, no criticism; 35–40 acceptable to most consumers; 25, unsuitable for consumption.

Symbols indicate: T., tallowy, and Ca., cabbage flavor and odor, respectively. Underlined numbers indicate samples of milk which developed pleasant almond-like flavor. *, milk tasted fresh at the end of 2 weeks of storage.

On the other hand the data presented in table 1–A indicate that the tallowy flavor could be quickly promoted by the addition of ascorbic acid to milk which was completely depleted of its total vitamin C content, providing it was treated with less than 0.8 ml. of H_2O_2 per liter shortly before pasteurization. It was obvious, therefore, that ascorbic acid either alone or with copper catalyzes the reaction which produces the tallowy flavor much more effectively than the copper alone. Conversely, when H_2O_2 treatment was applied to pasteurized milk, the products of ascorbic acid oxidation, such as dehydroascorbic acid and the residual H_2O_2, were retained in the samples,

The data on the effect of the addition of progressively increasing amounts of H_2O_2 to pasteurized milk upon the development of the tallowy flavor during its storage at low temperatures are presented in table 2. They show that in the case in which the volume of 30 per cent H_2O_2 added to milk was not in excess of that required to oxidize completely ascorbic acid to dehydroascorbic acid (at 0.021 ml.), the tallowy flavor was not induced by added copper. It also indicates that dehydroascorbic acid when present alone is not involved in the reaction responsible for the development of the tallowy flavor. However, it should be noted that from this point on with an increase in the volume of H_2O_2 added to milk up to 0.1 ml. per liter, inclusively, the catalysis of the tallowy flavor by copper followed an ecliptic path, with its high point at 0.051 ml. and the low ones at 0.028 and 0.102 ml., respectively, and that copper failed to promote it in the milk originally containing 0.204, 0.408 and 0.816 ml. of H_2O_2.

These results definitely indicate the existence of a critical region within which the oxidation of the lipid fraction of the milk with H_2O_2 is catalyzed by copper. Furthermore, the development of the tallowy flavor in the control portion of the milk treated with 0.051 ml. of H_2O_2 suggests the possibility that the favorable conditions for the copper catalysis of the tallowy flavor were established prior to its addition. In contrast to the milk described in table 1, the reaction was allowed to continue because of the presence of dehydroascorbic acid and of the residual H_2O_2. Undoubtedly, the development of the tallowy flavor in both the control and the portion of milk with copper and treated with 0.051 ml. of H_2O_2 was not a mere coincidence. It indicates that, under these conditions, dehydroascorbic acid may take part in the reaction resulting in the breakdown of the lipid fraction of the milk.

For the foregoing reason it was necessary to corroborate the results by an experiment in which dehydroascorbic acid was not a factor. This was done by the addition of 0.03, 0.06, 0.09 and 0.12 ml. of 30 per cent H_2O_2 per liter to portions of milk which was depleted of its total vitamin C content in the presence of 0.03 ml. of added H_2O_2 and the following heat treatment that takes place in pasteurization. The reaction which produces the tallowy flavor was not stimulated by the addition of H_2O_2 to milk completely depleted of its total vitamin C content. Whereas the development of the tallowy

TABLE 2

The relationship between the amounts of 30 per cent hydrogen peroxide added to milk after pasteurization, vitamin C content, added copper and the development of the tallowy flavor in milk subsequently held at 0 to 5° C.

Held at 0 to 5° C.	Unheated control sample		Ml. of 30% hydrogen peroxide per liter of pasteurized milk																							
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After pasteurized and H_2O_2 added	16.1	17.4	13.4	17.4	10.0	16.8	8.7	16.4	5.4	15.7	0.0	14.4	0.0	15.1	0.0	13.4	0.0	14.1	5.4	13.7	8.7	13.0	11.0	12.0	13.4	13.4
1	12.7	16.7	8.7	13.7	6.0	12.0	3.7	9.7	1.3	7.3		6.0		4.7		4.0		3.7		0.7		0.0		0.0		
3	7.3	12.0	3.0	8.4	1.7	6.3	0.7	5.3	0.0	3.0		3.0		0.0		2.3		2.0		0.0		0.0				
	Flavor score																									
1	40		40		40		40		40		40		42		40		40?		40		40		40		40	
3	37–T.		25–T.		25–T.		25–T.		38–T.		40		42		40		36–T.		40		40		40		40	
5	25–T.								39–T.?		42		42		40?		38–T.		40		40		40		40	
7									39–T.?		42		42		40		25–T.		39–T.?		40		40		40	
	Copper added 0.1 p.p.m.																									
1	7.3	14.7	3.7	10.7	2.0	9.0	1.0	8.7	0.0	8.0	0.0	5.7	0.0	4.3		4.0		3.7		1.3		1.0				
3	0.0	7.3	0.0	5.0	0.0	4.0	0.0	4.7		3.0		3.0		0.0		2.0		1.3		0.0		0.0				.
	Flavor score																									
1	29–T.		25–T.		25–T.		25–T.		29–T.		40		40		40		36–T.		40		40		40		40	
3	25–T.								29–T.		42		40		36–T.		25–T.		40		40		40		40	
5	.. . .				.		. .		36–T.		42		40		32–T.				32–T.		40		40		40	
7	. .				•				32–T.		42		38–T.		25–T.				25–T.		40		40		40	

Flavor scores and symbols—see footnote of Table 1–A.

flavor induced by copper followed an ecliptic path again, thus confirming the existence of a critical region for the reaction involving H_2O_2, copper catalyst, and the lipids of milk. The limits of the critical region are probably determined by the availability of the residual H_2O_2 at the end of the first reaction, on one side, and, on the other, by the inhibitory chemical changes produced in the milk by the addition of excessive amounts of H_2O_2. Of course a relationship might exist between the amounts of H_2O_2 added to milk and the catalytic properties of the copper.

Furthermore, in order to ascertain whether or not the sensitivity of milk to copper-induced tallowy flavor was affected by H_2O_2 treatment, portions of milk used in the preceding experiment were depleted of their total vitamin C content by heat in the presence of 0.03, 0.06, 0.09, 0.12 and 0.15 ml. of H_2O_2. Each sample was pasteurized immediately after the H_2O_2 was added. Through out the duration of the experiment (9 days) these samples behaved approximately the same way as the similar ones described in table 1. The promotion of the tallowy flavor was again found to be slow and erratic. Only one sample which was treated with 0.09 ml. of H_2O_2 prior to pasteurization developed the tallowy flavor, and then not until the seventh day of the holding period.

In conclusion it should be noted that in the case in which the amounts of H_2O_2 added to milk were in excess of those required to oxidize the ascorbic acid of the milk, especially after pasteurization, a rather pleasant almond-like flavor developed in the samples. Its presence was restricted to the area within which the tallowy flavor was induced by copper ecliptically (table 2), and its intensity followed essentially the same path.

Barron, DeMeio, and Klemperer (1) postulated that during ascorbic acid oxidation by atmospheric oxygen with the cupric ion as a catalyst, the metallic ion is reduced to cuprous form, and that the H_2O_2 is formed during the subsequent reoxidation of the catalyst. This by-product of the reaction is then readily split into water and oxygen. Their results show an uptake of one atom of oxygen per molecule of substrate at different pH values from 4.17 to 6.60. Hand and Chase (3) reported, however, an uptake of 1.19 and 1.85 atoms of oxygen per molecule of substrate at pH value of 6.85 (slightly higher than that of average milk) with copper and light as catalysts, respectively. They thought that the differences between copper and light in their effects on the oxygen combining power of vitamin C can be accounted for by the assumption that copper catalyzes the oxidation of ascorbic acid by H_2O_2. At the same time, Steinman and Dawson (12), using, pyrophosphate buffer, observed that, at a pH value of 6.3, the oxygen-combining power of vitamin C decreased when oxidized aerobically by ionic copper in the presence of catalase. These authors concluded that their results offer convincing evidence of H_2O_2 formation in the cupric oxidation of ascorbic acid.

Consequently Olson and Brown (10) attributed the development of the oxidized flavor in washed cream containing both ascorbic acid and copper (added) to the reaction involving phospholipid oxidation by H_2O_2 formed according to the scheme proposed by Barron and his associates. It should be born in mind, however, that by the numerous dilutions and reseparations these authors changed completely not only the multi-system of milk plasma, but also the physical properties of the cream, whereas the amounts of ascorbic acid which were added to the washed cream were those which some investigators think prevents oxidized flavor. Furthermore, their results show that at the end of the experimental trial (72 hours' duration period) the oxidized flavor developed in washed cream containing ascorbic acid, but no added copper. Obviously, the development of the oxidized flavor in the sample of cream just described could not be explained by the assumption that the free H_2O_2 was formed as well in the sample without copper, especially if consideration is given to the fact that the tallowy flavor was not induced by H_2O_2 when added alone to washed cream or to milk depleted of its total vitamin C content by the rapid oxidative method, or under certain conditions, to milk containing ascorbic acid. Neither could the promotion of the tallowy flavor in milk by partial oxidation of ascorbic acid to dehydroascorbic acid in the presence of added H_2O_2 be attributed to direct reaction between the agent and the lipids of milk.

Although the evidence presented in the preceding paragraphs definitely indicates that the oxidation of the lipid fraction of the milk by H_2O_2 could be catalyzed by copper, nevertheless it was evident that the reaction might take place only after all of the ascorbic acid is oxidized. Thus, if the H_2O_2 was formed during ascorbic acid oxidation by atmospheric oxygen with copper as a catalyst, it would be undoubtedly used to promote ascorbic acid oxidation first. As a matter of fact, we observed that the addition of H_2O_2, in amounts required to oxidize ascorbic acid completely, to milk previously treated with 0.1 p.p.m. of added copper, did not result in a faster promotion of the tallowy flavor, when compared with milk to which copper was added after all of the ascorbic acid were oxidized. In both milks the reaction was largely dependent upon the presence of the residual H_2O_2 at the point when all of the ascorbic acid was oxidized. Because of this, and in spite of the indirect evidence showing that H_2O_2 is formed during ionic copper oxidation from cuprous to cupric forms (aerobic) (3, 12), it would be reasonable to assume that free H_2O_2 is not formed in the milk during ascorbic acid oxidation by atmospheric oxygen with copper catalyst.[1]

These observations, together with the knowledge that the addition of 0.1 p.p.m. of copper to fresh pasteurized milk invariably results in the stimulation of the tallowy flavor, suggest that the reaction resulting in the development of tallowy flavor is a coupled reaction which is initiated when a certain equilibrium between the two forms of vitamin C has been established. Copper as an accelerator of ascorbic acid oxidation to dehydroascorbic acid might thus help to bring the system more rapidly to its critical point.

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[1] In a recent paper, L. W. Mapson noted that although the chloride ions accelerate the rate of reduction of Cu^{++} by ascorbic acid, they inhibit the oxidation of Cu^{+} by O_2 (13).

It has already been remarked that the physical state of washed cream differs from that of normal cream. With each successive washing and re-separation, the fat emulsion becomes less and less stable and easily breaks down, resulting in a partial reversal of the phases. Because of this a continious fat phase film might form at the surface of the cream. It would affect the rate of diffusion of atmospheric oxygen into the sample, as well as the rate of ascorbic acid oxidation. Consequently, the protective influence extended by the addition of 610 mg. of ascorbic acid to washed cream, as the data of Olson and Brown show (10), could be attributed to the exhaustion of the oxygen in the form of air which was incorporated into the cream by mechanical manipulations prior to the establishment of a critical equilibrium between the two forms of vitamin C. Thus the three-day holding period was not long enough to warrant a definite conclusion, since during that time the oxidized form of vitamin C was undoubtedly destroyed faster than it could be renewed by the oxidation of ascorbic acid.

It is of importance to note that ascorbic acid oxidation is responsible for the inactivation of several biologically active compounds (9, 11, 12), and that depending upon the environmental conditions the oxidation or inactivation of the other components of the system is either stimulated or prevented. Moreover, not all of the secondary reactions can be attributed to the activity of H_2O_2 formed during ascorbic acid oxidation. In their study of the ascorbic acid, ascorbic acid-oxidase, and oxygen system, Steinman and Dawson (12) and Powers and Dawson (11) have observed that the inactivation of the oxidase during the course of the reaction is largely prevented by the addition of catalase and peroxidase. The latter authors presented convincing evidence to show that the reaction which causes the inactivation of the oxidase is not due to the H_2O_2 formed in the reaction, but to some other factor, despite the fact that both catalase and peroxidase when added to the system markedly protect the enzyme against inactivation. McCarty (9), in his study of the inactivating action of ascorbic acid on the substance which induces transformation of pneumococcal types, came to the conclusion that this action of ascorbic acid (0.010 M) results from autoxidation, because the cupric ion markedly enhances the inactivating action of ascorbic acid (0.001 M) which when present alone in smaller concentration causes only partial inactivation of the substance. The cupric ion by itself did not alter the activity, whereas the effect of preformed H_2O_2 was found to be low. It was necessary to add a considerably higher concentration of H_2O_2 than is liberated in the course of the minimally effective amounts of ascorbic acid in order to bring about an inactivating effect comparable to that achieved by ascorbic acid.

Milk is a multi-component system containing biologically active materials. The activity of one of them, namely milk lipase, was shown to be dependent on the environmental conditions (temperature changes, Cu, oxygen) (6, 7, 8). It is possible, therefore, that however small the changes in the environmental conditions produced by chemical or physical means (exposure to light, addition of H_2O_2 and Cu, oxygen tension, etc.) might result in the stimulation of the activity of some of the biological agents present in the milk, and in the inactivation of the others. The environmental conditions would also determine the trend, extent and the magnitude of the reactions involved.

SUMMARY

1. It has been demonstrated that ascorbic acid oxidation is an essential link in the chain of the reactions resulting in the development of the tallowy flavor in milk, and that apparently the oxidation of the lipid fraction of the milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic and dehydroascorbic acids has been established.

2. The evidence is presented to show that a promoter (an enzyme) of ascorbic acid oxidation by H_2O_2 might be responsible for its quick conversion to dehydroascorbic acid, and that it is not the free H_2O_2 formed in the course of ascorbic acid oxidation which causes the breakdown of the lipid fraction of the milk, resulting in the development of the tallowy flavor. This view is supported indirectly by the observations indicating that H_2O_2 formed in the reaction would be used to oxidize the residual ascorbic acid as in the milk to which it was added; and that in the absence of a suitable catalyst the addition of H_2O_2 to milk completely depleted of its total vitamin C content does not result in the development of the tallowy flavor.

3. The reaction which produces the tallowy flavor is catalyzed by added copper in the presence of H_2O_2, providing the agent was added to milk completely depleted of its total vitamin C content by rapid oxidative method, or the amount of the agent added was in excess of that required to oxidize its ascorbic acid content. However, under the experimental conditions the catalysis of the tallowy flavor by copper were confined in a critical region, the limits of which were apparently determined by H_2O_2 concentration.

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A SIMPLIFIED EXTRACTION-DISTILLATION METHOD FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS OF CHEESE*

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A simple method for determining the volatile fatty acid content of cheese would be a valuable tool for the dairy chemist concerned with the ripening of cheese. Hiscox, Harrison, and Wolf (4, 5, 6) discussed this problem and proposed a method based on water extraction of the fatty acids from the cheese, and ether extraction of the fat and fat-soluble fatty acids with subsequent removal by washing the ether with dilute alkali. The acids in the extractions were then liberated by acidifying, and distilling with steam. The objections to this method are the great amount of time necessary to make a determination and the fact that a special glass-joined steam still of empirical dimensions is required.

All the volatile fatty acids are very soluble in ether and all except the short-chain acids are relatively insoluble in water. It seemed probable, therefore, that if cheese could be put into an acid solution consisting of dissolved, emulsified, and suspended materials, the fatty acids could be readily extracted by the use of ether. The volatile fatty acids can also be distilled quantitatively from an aqueous solution in an ordinary flask by use of a soluble salt, such as magnesium sulphate, to raise the boiling point, as shown by Friedemann (2). In determining the free volatile acids of butter, Ferris, Redfield and North (1) used a regular Kjeldahl apparatus in their distillation procedure. It was felt that if these principles could be successfully applied to cheese, a shorter, more rapid method requiring no special apparatus could be developed to determine the volatile acidity.

The object of this work was to attempt to develop a more rapid method and to observe more critically the retentive effect of nonvolatile fatty acids upon distillation of volatile fatty acids.

EXPERIMENTAL RESULTS

Simplified Extraction-Distillation Method

Preparation and extraction of cheese. In the method as finally adopted, a 10-gram representative sample of cheese weighed on a Torsion balance (sensitivity 4 mg.) was ground in a mortar with warm 10 per cent phos-

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phoric acid (50–55° C.). The ground cheese was washed quantitatively from the mortar into a 250-ml. centrifuge bottle until a total of 50 ml. of phosphoric acid solution was used. Ten ml. of ethyl alcohol and about 35 grams of $MgSO_4.7H_2O$ were added to the bottle and the mixture shaken. Fifty ml. of ethyl ether were then added to the bottle and the contents shaken vigorously for about 20 seconds. The pressure should be released carefully to avoid the stopper blowing out and spilling some of the contents. This bottle was then centrifuged one to two minutes at 2000 r.p.m. The ether layer was drawn off into a suction flask by means of a water-suction pump and the suction line was rinsed with 10 ml. of fresh ether. Two more extractions were carried out in the same manner except that no additional alcohol or $MgSO_4$ was added and that 35-ml. portions of ether were sufficient for each of the last two extractions. The ether was then transferred from the suction flask to a separatory funnel. Twenty-five ml. of N/10 NaOH were used to rinse out the suction flask and were then added to the contents of the funnel. The funnel was gently agitated with a rotary motion. Too vigorous shaking results in an emulsion which makes it difficult to separate the layers. The aqueous layer was drawn off into a 300-ml. Erlenmeyer flask. The ether layer was washed five additional times with 25 ml. of N/10 NaOH until 150 ml. of alkali had been used. By carefully heating on an electric hot plate or steam bath, the ether was driven from the combined alkaline washings in less than one hour.

Distillation and titration of the residue. While the ether was being driven off the alkaline washings, the residual material in the centrifuge bottle was transferred to an 800-ml. Kjeldahl flask. A total of exactly 230 ml. of distilled water was then added, part of it being used to rinse the centrifuge bottle. The solution, already acid due to the presence of phosphoric acid, was refluxed over a low flame on a wire gauze square three to five minutes to drive off the carbon dioxide. After slight cooling exactly 20 ml. of freshly boiled distilled water were used to rinse down the refluxer. The flask was placed on an asbestos board with a three-inch opening on an ordinary Kjeldahl distillation apparatus except that the traps were replaced by glass tubes of 10 mm. inside diameter to reduce refluxing to a minimum. A couple of glass beads were found essential to prevent bumping toward the end of distillation. The distillate was collected through Whatman No. 2 paper until 280 ml. had been obtained. It is imperative that not more than 280 ml. be collected because burning of the remaining contents will produce erratic results. The distillate was titrated with N/10 NaOH, using phenolphthalein as indicator. The funnel containing the filter paper was left in position for the second distillation involving the alkali washings.

Distillation and titration of the alkali washings of ether. The ether-free alkali washings were transferred to an 800-ml. Kjeldahl flask using three 15-ml. portions of distilled water to rinse the flask. About 35 grams of

$MgSO_4.7H_2O$ were added and enough 50 per cent H_2SO_4 to adjust to pH 2, using pHydrion paper as indicator. Refluxing and distillation procedures were followed similar to that used with the residual material except that the distillation was continued until the contents of the Kjeldahl flask started to crystallize. This can be readily detected when the material in the flask becomes murky. It is important not to heat the flask after crystallization because the H_2SO_4 will then decompose and give erroneous results. The distillate was passed through the same condenser tube and was filtered through the same paper used for the first distillation and collected in an Erlenmeyer flask. It was titrated directly.

Neutral alcohol was used to rinse the insoluble acids from the condenser. The rinsings were caught in the funnel with the filter paper which had retained the remainder of the insoluble acids during the two distillations. These alcohol rinsings were titrated.

The sum of the titrations of the first and second water distillates and of the alcohol rinse was considered the volatile acidity of the cheese. Results were expressed as ml. N/10 acid per 100 grams of cheese.

A COMPARISON OF METHODS

In order to critically evaluate the reliability of this improved technique, more aptly called the simplified extraction-distillation method, the results from a number of cheeses were compared to the standard Hiscox method and in some cases to the direct steam distillation method. The direct steam distillation was made by distilling 500 ml. from 20 gm. of cheese made up to 100 ml. with CO_2-free water. Table 1 gives the results from eight Cheddar cheeses covering a wide range of volatile acids. The simplified extraction-distillation method compared very favorably with the Hiscox method. The direct steam distillation method gave low results for all cheeses, this being characteristic of the method as shown previously by Hiscox, Harrison, and Wolf (5).

Further analyses were extended to cover various other types of cheese. Table 2 shows the results from seven different types of cheese. In each instance the results from the simplified extraction-distillation method were in very good agreement with those of the Hiscox method.

The Retention of Volatile Fatty Acids by Nonvolatile Fatty Acids

In his work on the determination of volatile alcohols and acids Friedemann (2) observed that short chain fatty acids, such as acetic, butyric, caproic, and caprylic could be quantitatively recovered by direct distillation in the presence of $MgSO_4$. An experiment was conducted to observe the effect of $MgSO_4$ upon the percentage recovery of fatty acids during direct distillation, using all acids in the series from butyric through myristic.

A quantity of pure fatty-acid solution was added to a clean 800-ml.

TABLE 1

*A comparison of three different methods in the determination of volatile fatty acids in Cheddar cheeses**

Cheese	I Direct steam			II Hiscox							III Simplified extraction-distillation				
	Combined		Total	Water phase		Fat phase		Residue		Total	Ether extract		Residue		Total
	Sol.	Insol.		Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		Sol.	Insol.	Sol.	Insol.	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
1	5.2	0.5	5.7	4.9	0.6	2.0	1.5	2.9	2.7	14.6	9.6	2.9	2.0	0.7	15.2
2	74.5	1.3	75.8	72.5	1.7	4.6	5.5	4.3	5.3	93.9	72.0	8.2	12.5	1.1	93.8
3	35.8	1.4	37.2	37.6	1.6	2.7	4.4	2.2	3.1	51.6	33.2	6.5	7.5	0.4	47.6
4	11.5	4.0	15.5	13.9	0.9	2.3	1.6	1.4	2.5	22.6	12.1	3.9	6.1	2.7	24.8
5	27.4	0.5	27.9	28.4	0.6	3.2	1.1	2.2	1.7	37.1	25.9	3.2	5.6	1.6	36.3
6	35.5	0.8	36.3	39.7	0.4	1.3	1.5	2.6	1.6	47.1	34.8	4.9	7.9	1.7	49.3
7	44.0	0.9	44.9	46.9	1.1	2.1	5.1	4.0	2.0	60.6	38.2	6.3	15.0	2.3	61.8
8	28.9	0.8	29.7	29.8	1.1	3.3	3.0	2.9	1.0	41.5	28.9	5.6	5.1	0.8	40.4
Average			34.1							46.1					46.2

* All values expressed as ml. N/10 acid per 100 grams cheese.

TABLE 2

*A comparison of the Hiscox method and the simplified extraction-distillation method in the determination of volatile fatty acids in various types of cheese**

Type of cheese	Hiscox							Simplified extraction-distillation			
	Water phase		Fat phase		Residue		Total	Ether extract	Residue	Combined	Total
	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		Sol.	Sol.	Insol.	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
1. Blue	13.0	6.5	2.5	9.0	2.0	4.5	37.5	19.7	8.5	12.2	40.4
2. Switzer	39.6	1.3	2.0	1.8	1.4	1.0	47.1	38.0	7.7	3.8	49.5
3. Chantelle	22.9	0.7	1.4	1.0	2.4	1.5	29.9	22.2	6.4	2.8	31.4
4. Camembert	23.0	5.7	2.2	8.1	†	†	39.0	27.0	4.5	8.4	39.9
5. Snappy cheese food	14.9	1.1	3.2	4.5	4.7	1.2	29.6	19.9	5.3	5.6	30.8
6. D'Oka (Trappist)	37.0	2.8	4.8	6.8	†	†	51.4	36.9	4.6	7.7	49.2
7. Leiderkrantz	89.0	2.1	4.7	4.2	2.2	1.5	103.7	84.3	13.7	4.8	102.8
Average							48.3				49.1

* All values expressed as ml. N/10 acid per 100 grams cheese.

† Residue washings combined with washings from fat and both distilled over at same time.

Kjeldahl flask and titrated with N/10 NaOH to get the exact amount of acid present. About 300 ml. of H_2O were added and enough H_2SO_4 to lower the pH to 2.0. Approximately 35 grams of $MgSO_4.7H_2O$ were added and the solution distilled to the point of crystallization. The distillate was titrated and the recovery calculated.

Table 3 shows the percentage recovery of the fatty acids under the stated conditions. The results confirm the conclusions of Friedemann that distillation with $MgSO_4$ will quantitatively recover acetic, butyric, caproic, and caprylic acids. In addition, these data (table 3) show that caproic and lauric acids can also be quantitatively recovered under similar conditions. Myristic acid was not volatile enough even under the elevated temperature provided by $MgSO_4$ to be completely distilled, but was about 70 per cent recovered.

TABLE 3

The percentage recovery of fatty acids by direct distillation with $MgSO_4$ present in the distillation mixture

Fatty acid	Percentage recovery
Acetic	100
Butyric	100
Caproic	100
Caprylic	100
Capric	100
Lauric	100
Myristic	71

The known retentive rôle played by fat during distillation does not concern itself in the simplified extraction-distillation and Hiscox methods as there is no fat present in the distillation mixtures. However, such nonvolatile acids as palmitic and oleic, if present, are carried over into the distillation mixture. If these nonvolatile acids exert a retentive effect upon the volatile fatty acids it would be important to establish this fact. The following experiment was designed to furnish information on this subject.

Quantities of tenth normal volatile fatty acids, ranging from 2.0 to 6.0 ml. amounts, were added to a specific quantity of palmitic or oleic acid (0.25 and 0.50 gram, respectively) in 300 ml. of distilled water. Approximately 35 grams of $MgSO_4.7H_2O$ were added to the mixture followed by the addition of 50 per cent H_2SO_4 until a pH of 2 was obtained. After distilling the acid mixture to the point of crystallization the distillate was titrated and percentage recovery of the fatty acids calculated.

TABLE 4

The effect of nonvolatile fatty acids upon the recovery of individual volatile fatty acids during direct distillation with $MgSO_4$

Volatile acids	0.25 gram palmitic acid in distillation mixture	0.5 gram oleic acid in distillation mixture
	Percentage recovery	Percentage recovery
Butyric (C_4)	100.	100.
Caproic (C_6)	100.	100.
Caprylic (C_8)	93.2	92.8
Capric (C_{10})	84.4	79.1
Lauric (C_{12})	71.1	53.6
Myristic (C_{14})	36.5*	20.6*

* Approximately 70.0 per cent myristric acid is recovered during direct distillation with $MgSO_4$ and no nonvolatile fatty acid.

The nonvolatile acids, palmitic and oleic, exerted a retentive effect on some of the higher chain fatty acids during distillation. However, for those fatty acids which are mostly concerned with the volatile acidity of cheese the effect of nonvolatile acids was negligible. Table 4 shows that fatty acids up through caproic are unaffected during distillation by nonvolatile acids, while from caprylic to myristic there is a varying effect as the percentage recovery ranges from 93.2 to 20.6 per cent. The percentage recovery is indirectly related to the number of carbon atoms present in the volatile fatty acids, a relationship which is clearly shown in table 4.

DISCUSSION

The advantages of the simplified extraction-distillation procedure become very apparent when such factors as economy of time and utilization of regular apparatus are considered. It takes approximately four hours to complete an analysis on one cheese sample using the new procedure, whereas the

Hiscox method requires well over forty hours. The use of the Kjeldahl distillation apparatus eliminates any great delay which might occur in case of breakage with special glass stills, and, because of its efficient design, enables the laboratory technician to greatly increase the number of samples that can be handled at one time.

In the actual method, phosphoric acid was chosen because it is a good cheese solvent. The purpose of the alcohol was to afford better contact between the ether and water when the extraction was carried out. In the early portion of the method $MgSO_4.7H_2O$ was added because it allowed the cheese proteins to float on the aqueous layer as a crust after centrifuging, greatly facilitating the separation of the ether from the water. It also increased the efficiency of the extraction, probably because it lowered the solubility of the ether in the water phase. In the latter portion of the method $MgSO_4$ was used to raise the boiling point of the solution.

During the distillation of the residue the turbidity of the solution, as a result of the presence of proteins, prevented easy observation of crystallization. Hence, this distillation was conducted on a volume basis. In the case of the alkali washings of the ether extract there was no turbidity and consequently the distillation could be carried to crystallization.

Washing of free fatty acids from an ether fat solution was tried in accordance with the directions of Hiscox, Harrison and Wolf (5) except that six 25-ml. portions of N/10 NaOH were used instead of six 50-ml. portions as these authors suggested. Known amounts of volatile fatty acids were dissolved in pure fresh butter oil and the solution added to ether in a separatory funnel. The ether solution was then washed with the six portions of dilute alkali. When the washings were acidified and distilled in the presence of $MgSO_4$ it was found that all the volatile acids tried from butyric up to and including lauric were recovered completely. This also confirms the work of Gould and Johnson (3) who found that dilute alkali will remove practically all the fatty acids from a rancid fat. The results obtained on fat blanks alone were the same as the direct titration of the fat showing little or no saponification due to the alkali.

The retentive effect of the nonvolatile acids is probably not as great as the effect of butter fat. Hiscox and Harrison (4) recovered 95, 54, and 19 per cent of caprylic, capric and lauric acids, respectively, in the presence of butter fat. Our results show 93, 84, and 70 per cent recovery for these same acids in the presence of palmitic acid. These results are not entirely comparable due to the great differences in the distillation techniques used. They do, however, show that in either the method presented here or in the technique of Hiscox, Harrison, and Wolf (5) there is still a retentive factor present. However, since most of the volatile acidity is due to short-chain acids, the retentive effect of the nonvolatile acids during the distillation is of minor importance.

Hiscox and Harrison (4) distilled over 90 to 100 per cent of the volatile fatty acids in the presence of cheese proteins so the retention of fatty acids by proteins was much less marked than by the fat. It appears obvious in our work that the retention of volatile acids by the proteins in the residue did not present any appreciable error as only a relatively small amount of fatty acids was carried in the residue and practically all of this was recovered during direct distillation.

Recovery experiments were performed to see if the ether extraction of the acid cheese mush was efficient. Known amounts of the volatile fatty acids were added to the acid cheese mush and the regular procedure. previously described, was followed. Results indicated that about 95 per cent of the butyric acid added was recovered by three extractions, while about 85 per cent of added lauric was recovered. Further extractions failed to recover significant amounts of volatile acids. However, in actual analyses on a number of cheeses it was noted that ether extraction alone gave significantly lower results than those obtained with the Hiscox method. An increase in the number of ether extractions with increased amounts of ether did not remedy this situation, although using four 50-ml. portions of ether instead of the customary three did recover more of the volatile acids. It was not until the residue was distilled and the titration value added to that of the ether extraction that a very close agreement with the Hiscox method was obtained.

SUMMARY

A method for determining the volatile fatty acids of cheese is described. The method, referred to as the simplified extraction-distillation method, is based on two distillations of acid cheese solution, one associated with the fat phase and the other with the residual material. After ether extraction of the acid-cheese mixture, the volatile fatty acids were removed from the ether with dilute alkali. The alkali rinses were heated to drive off the ether, then acidified, refluxed to remove carbon dioxide, and distilled in the presence of $MgSO_4$ until crystallization occurred. In the meantime, 250 ml. of distilled water were added to the residual material and the solution distilled until 280 ml. of distillate were collected. The sum of the titrations from the two distillations plus that of the alcohol rinse represented the total volatile acids of the cheese.

This new method and the method of Hiscox, Harrison, and Wolf (5) gave nearly identical results for a wide variety of cheeses. It has advantages over the latter method in that no special apparatus is necessary, more distillations can be accomplished in the course of a day, and the time required to analyze a sample of cheese is very greatly reduced.

The findings of Friedemann (2) that acetic, butyric, caproic, and caprylic acids can be quantitatively recovered by distilling in the presence of $MgSO_4$ were confirmed. In addition, capric and lauric acids were quantitatively

recovered under similar conditions, while myristic acid was incompletely recovered.

Nonvolatile fatty acids, such as palmitic and oleic, exerted a retentive effect on caprylic, capric, lauric, and myristic acids during direct distillation in the presence of $MgSO_4$ with the degree of retention being in direct relationship to the number of carbon atoms present in the volatile acids. When butyric and caproic acids were distilled, the nonvolatile acids exerted no retentive effect.

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DEFLUORINATED ROCK PHOSPHATE AS A PHOSPHORUS SUPPLEMENT TO THE RATIONS OF DAIRY CATTLE[1]

T. W. GULLICKSON AND FLOYD C. OLSON[2]
Minnesota Agricultural Experiment Station, St. Paul, Minnesota

The scarcity of steamed bone meal during the war period made it necessary to find a satisfactory substitute that could be used to supplement dairy herd rations that are deficient in phosphorus. Raw rock phosphate, the natural choice for such use, has been proven unsatisfactory because of its high fluorine content which renders it toxic. Within the last few years several methods have been perfected for removing the fluorine from natural phosphates to a level approximating that in bone meal, making the resulting products safe for feeding to cattle. Recently, however, Barrentine, Maynard, and Loosli (1), working with rats, observed considerable difference in the availability of the calcium and phosphorus in defluorinated rock phosphates produced by these methods. The Florida station (2) reported rather undecisive results regarding the comparative palatability of defluorinated superphosphate and bone meal for cattle, but no comparisons were indicated in regard to their values as sources of phosphorus.

The following report presents briefly the results obtained in trials in which a defluorinated rock phosphate[3] was compared with steamed bone meal in its ability to serve as a supplement to phosphorus-deficient rations of dairy cattle. The process followed in its manufacture was described as "the calcination of phosphate rock in addition to the use of acids all of which maintains the original ortho form of phosphate in the product." The product as fed compared favorably in composition with that of steamed bone meal. It contained approximately 29.8 per cent calcium and 14.0 per cent phosphorus. The fluorine content was under 0.10 per cent, which according to Mitchell (3) makes it safe, under all reasonable conditions for feeding to cattle.

EXPERIMENTAL

Four grade Holstein heifers, E416, E418, E426 and E441, all about 21 months old when started, were used in the trials. The experiment lasted 17 months, the first six months of which was a preparatory period during which all animals were fed similar phosphorus-deficient rations. During the last eleven months, the experimental period, the same plan of feeding as before was followed except that bone meal was added as a supplement to the

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[2] Resigned July 1, 1945.

[3] Supplied by International Minerals and Chemical Corporation, Chicago, Illinois.

rations of E418 and E441, respectively, and E416 and E426 received equivalent additions of phosphorus to their rations in the defluorinated rock phosphate. Each ration with supplements added was designed to provide adequate protein but slightly less than the required amount of phosphorus. The mineral supplements were added directly to the grain ration at each feeding.

All heifers were fed twice daily in mangers designed for cattle on experiment and records were kept of amounts of feedstuffs and supplements fed and consumed. Chemical analysis, including calcium and phosphorus determinations, was made of all feeds and supplements fed. The inorganic phosphate content of blood plasma and the weight of each animal was determined regularly every 30 days. Observations relating to their physical condition, general appearance, evidence of pica and anorexia, were made daily. Heifers, when in the barn, were stanchioned in comfortable stalls equipped with drinking cups. When weather permitted, they spent from two to six hours daily outdoors in a grass-free yard. Results were measured in terms of general appearance of animals, appetite shown for good-quality low-phosphorus content prairie hay, rate of gain in weight, and by the indicated concentration of inorganic phosphate of the blood plasma.

TABLE 1

Phosphorus intakes in ration and supplement, and blood plasma inorganic phosphorus concentration of each heifer during the preparatory and experimental periods

Animal No.	Kind of supplement	Phosphorus intake daily			Blood plasma inorganic phosphorus
		In ration	In supplement	Total	
		Preparatory period			
		grams	*grams*	*grams*	*mg.%*
E416	None	7.35		7.35	3.06
E426	"	7.44		7.44	3.38
Average		7.40		7.40	3.32
E418	None	7.23		7.23	2.82
E441	"	8.00		8.00	3.31
Average		7.62		7.62	3.07
		Experimental period			
		grams	*grams*	*grams*	*mg.%*
E416	Defluor. phosphate	7.40	2.79	10.19	4.40
E426	"	7.85	2.87	10.72	4.79
Average		7.63	2.83	10.46	4.60
E418	Bone meal	7.39	2.70	10.09	4.42
E441	"	8.61	2.80	11.41	5.52
Average		8.00	2.75	10.75	4.97

RESULTS AND DISCUSSION

Some of the more significant data relating to phosphorus intakes, weights and inorganic phosphate content of the blood plasma of all animals during the periods of the experiment are presented in tables 1 and 2.

The data presented in table 1 clearly shows that the basal rations fed were in all cases deficient in phosphorus. This is indicated by the subnormal concentration of inorganic phosphorus in the blood plasma of all animals during the preparatory period. During this period all heifers also exhibited marked pica, poor general appearance and indifferent appetite for hay as well as other characteristic symptoms typical in cattle suffering from a deficiency of phosphorus in their rations.

TABLE 2

Weights and average daily gains of heifers at various intervals during the preparatory and experimental periods

Animal No.	Weight at			Average daily gain from		
	Start	6th month	End	Start to 6th month	6th month to end	Start to end
			Preparatory period			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
E416	746	825	825	0.44		0.44
E426	795	880	880	0.47		0.47
Average	770	852	852	0.46		0.46
E418	765	832	832	0.37		0.37
E441	848	949	949	0.56		0.56
Average	806	890	890	0.47		0.47
			Experimental period			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
E416	825	857	1006	0.18	0.99	0.55
E426	880	959	1082	0.44	0.82	0.61
Average	852	908	1044	0.31	0.91	0.58
E418	832	885	1082	0.29	1.31	0.76
E441	949	1044	1199	0.53	1.03	0.76
Average	890	964	1140	0.41	1.17	0.76

As is indicated in table 1 the average blood plasma phosphate concentration in each animal was considerably higher during the experimental than for the preparatory period. This rise or increase in phosphate concentration occurred simultaneously and to approximately the same extent in all animals in both groups. The rise became apparent within 30 days after feeding of supplements was started, and although the concentrations fluctuated quite widely from month to month, the trend throughout was upward for all animals. All the heifers also showed marked improvement in their physical appearance and appetite for prairie hay, and pica which was exhibited by all animals at the start, gradually disappeared as the experimental period progressed. From these results and observations it must be concluded that the phosphorus supplied in the supplements was being utilized by the heifers.

It will be observed that heifers made only slightly greater average daily gain in weight while receiving the supplements than during the preparatory period. This is especially true of E416 and E426, the heifers that were fed defluorinated product. In this connection it should be explained that as appetite for prairie hay improved, somewhat less grain was fed in order to maintain the phosphorus content of the basal ration at the desired low level. Consequently, digestible nutrients intakes did not increase directly with volume of feed consumed. There was in fact a slight decline in nutrient intake during the first two or three months which accounts for the slower rate of gain indicated (table 2) for the first six months of the experimental period. During the last five or six months of this period, however, hay consumption increased greatly with consequent rise in energy intake and marked increase in rate of daily gain in weight. The higher average daily gains made by the heifers fed bone meal as compared to those fed defluorinated rock phosphate during a similar period of time is only slightly (if at all) significant. No difference in palatability of the two supplements was discerned during the progress of the trials.

SUMMARY AND CONCLUSIONS

A defluorinated rock phosphate was compared with steamed bone meal in its ability to serve as a satisfactory supplement to phosphorus-deficient rations of dairy cattle. Four grade Holstein heifers about 21 months of age were used. They were fed similar phosphorus-deficient basal rations during the 17-months period of the experiment. During the last eleven months two of them were fed steamed bone meal as a supplement to their rations; the other two received equivalent additions of phosphorus from a defluorinated rock phosphate product. Total phosphorus intakes were kept slightly below the requirements of each animal.

Under the conditions of the experiment no significant difference was observed between defluorinated rock phosphate and steamed bone meal in availability of their phosphorus to dairy cattle. Animals fed the bone meal made slightly greater gains in weight. No difference was noted in the palatability of the two supplements as fed.

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(1) Barrentine, B. F., Maynard, L. A., and Loosli, J. K. The Availability of the Calcium and Phosphorus of Defluorinated Rock Phosphate for the Rat. Jour. Nutr., 27: 35–42. 1944.

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(3) Mitchell, H. H. The Use of Phosphorus-containing Substitutes for Bone Meal in Livestock Feeding with Particular Reference to the Fluorine Hazard. Nat. Res. Council Comm. on Anim. Nutr. Rept. No. 10. 1943.

PROGRAM

FORTY-FIRST ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

IOWA STATE COLLEGE
AMES, IOWA
JUNE 18–20, 1946

PROGRAM COMMITTEE

GENERAL:

A. C. RAGSDALE, *Chairman,*
University of Missouri

G. W. SALISBURY,
Cornell University

FLOYD ARNOLD,
University of Maryland

G. C. NORTH, Beatrice Creamery,
Chicago, Illinois

F. E. NELSON,
Iowa State College

MANUFACTURING:

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Beatrice Creamery

B. E. HORRALL,
Purdue University

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Iowa State College

EXTENSION:

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University of Maryland

W. T. CRANDALL,
Cornell University

E. H. LOVELAND,
University of Vermont

FLOYD JOHNSTON,
Iowa State College

PRODUCTION:

G. W. SALISBURY, *Chairman,*
Cornell University

DWIGHT ESPE,
Iowa State College

DWIGHT SEATH,
Louisiana State University

REGISTRATION

MEMORIAL UNION

IOWA STATE COLLEGE

Meetings will be held in buildings on the campus of Iowa State College and headquarters will be in the Memorial Union.

SCHEDULE OF PROGRAMS

Date and Time	General	Production	Extension	Manufacturing
Tuesday *June 18, 1946*				
8: 00	Registration			
9: 30–12: 00	Opening Session			
1: 00– 4: 00		Section A Section B	Section	Section
4: 00– 5: 00	Committees	Committees	Committees	Committees
7: 30	Reception (Informal)			
Wednesday *June 19, 1946*				
9: 00–12: 00		Joint Business and Symposium		Symposium
1: 00– 3: 00		Section A Section B	Section	Section
3: 00– 4: 00		Business	Business	Business
4: 00– 5: 00	Committees Campus Tours	Committees Campus Tours	Committees Campus Tours	Committees Campus Tours
7: 30	Mixer			
Thursday *June 20, 1946*				
7: 00	Southern Section Breakfast			
9: 00–11: 00		Joint Symposium		Section
11: 00–12: 00		Business	Business	Business
1: 00– 3: 00		Section A Section B	Section	Section
3: 00– 5: 00	Business			
6: 30	Banquet Installation of Officers, Borden Awards			

COMMITTEE MEETINGS

Suitable rooms will be available for all committees and for other groups which may desire to meet. Elmer Hansen will have charge of room assignments for the Extension and Production Sections and F. E. Nelson for the Manufacturing Section.

PROJECTION EQUIPMENT

Lanterns will be available, upon request, for projection of standard and 2″ × 2″ slides. Projectors for 8 and 16 mm. movies also will be available by arrangement.

DAIRY FARM—HERD—MILKING PARLOR

All persons attending the meetings are invited to visit the Dairy Husbandry Farm which is located approximately three-fourths of a mile south of the campus. The milking parlor will be in operation from 4: 00–6: 00 both morning and afternoon.

GENERAL PROGRAM

Tuesday, June 18, 1946

8:00 Registration, Memorial Union.

9:30–12:00 Opening Sessions, *Great Hall, Memorial Union.*

C. A. Iverson, *Head, Department of Dairy Industries,* Presiding.

Introduction of Officers and Guests.

Address of Welcome—Dr. Charles E. Friley, *President, Iowa State College.*

Presidential Address—J. A. Nelson, *President, American Dairy Science Association.*

Guest Speaker—E. S. Estel, *Editor, Creamery Journal; Manager, Dairy Cattle Congress; Secretary, Iowa State Dairy Association.*

Announcements.

1:00– 4:00 Sectional Meetings.

Production Section A, Vitamins, *Room 19, Agricultural Hall.*

Production Section B, Hormones, Blood, and Disease, *Agricultural Assembly Room, Agricultural Hall.*

Manufacturing Section, Dry Milk Products, *Room 118, Dairy Industries Building.*

Extension Section, D.H.I.A. Work, *Room 117, Agricultural Hall.*

4:00– 5:00 Committee Meetings.

7:30 Reception, *Women's Gymnasium.*

Wednesday, June 19, 1946

9:00–12:00 Sectional Meetings.

Manufacturing Section, Quality Symposium, *Room 118, Dairy Industries Building.*

Joint Business and Symposium Production and Extension Sections, Committee Reports—Breeds Relations, *Agricultural Assembly Room, Agricultural Hall.*

1:00– 3:00 Sectional Meetings.

Manufacturing Section, Cheese and Evaporated Milk, *Room 118, Dairy Industries Building.*

Production Section A, Thyroprotein, Protein, Minerals, *Room 19, Agricultural Hall.*

Production Section B, Feeding and Management, *Agricultural Assembly Room, Agricultural Hall.*

Extension Section, Artificial Breeding—Exhibits, *Room 117, Agricultural Hall.*

3:00– 4:00 BUSINESS MEETINGS OF SECTIONS.

Extension Section, *Room 117, Agricultural Hall.*

Manufacturing Section, *Room 118, Dairy Industries Building.*

Production Section, *Agricultural Assembly Room, Agricultural Hall.*

4:00– 5:00 COMMITTEE MEETINGS.

CAMPUS TOURS.

7:30 MIXER, *Great Hall, Memorial Union.*

Thursday, June 20, 1946

7:00 BREAKFAST-SOUTHERN SECTION, *Pine Room, Memorial Union.*

9:00–11:00 SECTIONAL MEETINGS.

Manufacturing Section, Bacteriology and Chemistry, *Room 118, Dairy Industries Building.*

Joint Symposium Production and Extension Sections, Herd Health and Sanitation, *Agricultural Assembly Room, Agricultural Hall.*

11:00–12:00 BUSINESS MEETINGS OF SECTIONS.

Extension Section, *Room 117, Agricultural Hall.*

Manufacturing Section, *Room 118, Dairy Industries Building.*

Production Section, *Agricultural Assembly Room, Agricultural Hall.*

1:00– 3:00 SECTIONAL MEETINGS.

Extension Section, 4-H Club Work, Quality Programs, *Room 117, Agricultural Hall.*

Manufacturing Section, Chemistry, Sanitation, Teaching, *Room 118, Dairy Industries Building.*

Production Section A, Hay, *Room 19, Agricultural Hall.*

Production Section B, Breeding, *Agricultural Assembly Room, Agricultural Hall.*

3:00– 5:00 GENERAL BUSINESS SESSION, *Agricultural Assembly Room, Agricultural Hall.*

6:30 ANNUAL BANQUET—INSTALLATION OF OFFICERS AND PRESENTATION OF BORDEN AWARDS.

SECTIONAL PROGRAMS

Tuesday, June 18

Afternoon Session, *Room 117 Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00– 1:15 **Opening Business Session.**

1:15- 4:00 **D.H.I.A. Work.**

- E1. Considerations in the Development of Dairy Herd Improvement Association Work—a panel discussion led by J. F. KENDRICK, *Bureau of Dairy Industry, U.S.D.A.*
- E2. More Accurate Records on More Cows Through County Testing Laboratories—J. E. STALLARD, *University of Wisconsin.*
- E3. Practices Used in Dairy Herd—Improvement Associations to Evaluate Pasture—R. B. BECKER, *University of Florida.*
- E4. Report of Testing Committee—W. T. CRANDALL, *Cornell University.*
- E5. Effective Use of Herd Records—R. E. HORWOOD, *Michigan State College.*

4:00- 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND GLENN SALISBURY—*Co-Chairmen*

9:00-12:00 **Joint Symposium Production and Extension Sections.**

Breeds Relations—Committee Reports.

- A. Pasture and Roughage Committee, R. B. BECKER, *Chairman, University of Florida.*
- B. Program of the Purebred Dairy Cattle Association, G. A. BOWLING, *Secretary.*
- C. Breed Relations Committee, H. A. HERMAN, *Chairman, University of Missouri.*
- D. Uniform Rules for Official Testing, FLOYD JOHNSTON, *Iowa State College.*
- E. Dairy Cattle Breeding Committee, C. L. BLACKMAN, *Chairman, Ohio State University.*
- F. Program Suggestions for Inter-Breed Groups, E. E. SCHEIDENHELM, *Rutgers University.*
- G. Round Table Discussion, BREED ASSOCIATION REPRESENTATIVES.

Afternoon Session, *Room 117, Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00- 3:00 **Artificial Breeding, Exhibits.**

- **E6.** Inspection of Exhibits, each exhibit will be explained by the person in charge.
- **E7.** Report of Exhibit Committee, ARTHUR R. PORTER, *Iowa State College.*

E8. Organization and Operation of Artificial Breeding Associations, C. A. HUTTON, *University of Tennessee.*

E9. Some New Management Methods for Artificial Breeding Units, E. J. PERRY, *Rutgers University.*

E10. Schools and Program for Training Inseminators, RAYMOND ALBRECTSON, *Cornell University.*

3:00– 4:00 **Business Session.**

4:00– 5:00 **Committee Meetings.**
Campus Tours.

Thursday, June 20

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND GLENN SALISBURY—*Co-Chairmen*

9:00–11:00 **Joint Symposium Production and Extension Sections.**
Herd Health and Sanitation—a panel discussion led by W. E. PETERSEN, *University of Minnesota.*

11:00–12:00 **Business Session**—*Room 117, Agr. Hall.*

Afternoon Session, *Room 117, Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00– 3:00 **4-H Club Work, Quality Programs.**

E11. New Developments in 4-H Club Work, J. C. NAGEOTTE, *Pennsylvania State College.*
  a. Discussion of 4-H Club Projects.

E12. Extension Work in Quality Improvement of Dairy Products, A. B. NYSTROM, *Bureau of Dairy Industry.*

E13. Minnesota's Dairy Quality Improvement Program, H. R. SEARLES, *University of Minnesota.*

3:00– 5:00 **General Business Session,** *Agr. Assembly Room, Agr. Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

MANUFACTURING PROGRAM

Tuesday, June 18

Afternoon Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

1:00– 4:00 **Dry Milk Products.**

M1. A New Quantitative Method for Determining the Solubility of Milk Powders, J. FRANK CONE, U. S. ASHWORTH, *State College of Washington.*

M2. Drying Milk by the Use of Cold, OTTO F. HUNZIKER, *Dairy Consultant, La Grange, Illinois.*

M3. Biochemical Changes in Whole Milk and Ice Cream Mix Powders During Storage, N. P. TARASSUK, E. L. JACK, *University of California.*

M4. The Role of Moisture and Oxygen Levels on the Keeping Quality of Dry Ice Cream Mix, R. W. KUNKEL, S. T. COULTER, AND W. B. COMBS, *University of Minnesota.*

M5. Factors Related to the Brown Discoloration of Powdered Whole Milk, WALTER KRIENKE AND P. H. TRACY, *University of Illinois.*

M6. Observations on the Relative Keeping Quality of Powdered and Frozen Whole Milk, P. H. TRACY, JOHN HETRICK, AND WALTER KRIENKE, *University of Illinois.*

M7. Physico-chemical Factors Affecting the Reconstitutability of Dry Whole Milk, G. H. WILSTER, OTTO SHREITER, AND P. H. TRACY, *University of Illinois.*

M8. Changes in the Fluorescence of Dry Whole Milk on Storage, R. JENNESS, S. T. COULTER, AND R. LARSON, *University of Minnesota.*

M9. Changes in Reducing Materials in Dry Whole Milk During Storage, L. K. CROWE, S. T. COULTER, AND R. JENNESS, *University of Minnesota.*

M10. Drying Milk in Nitrogen or Carbon Dioxide, S. T COULTER, *University of Minnesota.*

M11. Relation of the Temperature of Separation and Heat Treatment Given the Serum Solids to the Keeping Quality of Spray Dried Ice Cream Mix, HARRY PYENSON AND P. H. TRACY, *University of Illinois.*

4:00– 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

9:00–12:00 **Quality Symposium**—H. F. JUDKINS, *National Dairies, New York, Chairman.*

A. Milk.—G. M. TROUT, *Michigan State College;* C. V. CHRISTIANSEN, *Bowman Dairy Co., Chicago, Ill.*

B. Ice Cream.—A. C. DAHLBERG, *Cornell University;* E. C. SCOTT, *Swift & Company, Chicago, Ill.*
C. Butter.—R. V. HUSSONG, *University of Illinois;* G. W. SHADWICK, *Beatrice Creamery Co., Chicago.*
D. Cheese.—W. V. PRICE, *University of Wisconsin;* H. L. WILSON, *Kraft Cheese, Chicago, Ill.*
E. Manufactured Products.—P. H. TRACY, *University of Illinois;* O. F. GARRETT, *M & R Dietetic Laboratories, Columbus, Ohio.*

Afternoon Session, *Room 118, Dairy Industries Building*

G. C. NORTH, *Chairman*

1:00– 3:00 **Cheese and Evaporated Milk.**
M12. Gas Packing of American Cheddar Cheese, N. S. GOLDING, J. N. REYNOLDS, AND C. C. PROUTY, *State College of Washington.*
M13. The Action of the Associate Starter Organisms in American Cheddar Cheese, C. C. PROUTY AND N. S. GOLDING, *State College of Washington.*
M14. Studies on the Resistance of Various Cheese Cultures to the Action of Bacteriophage, F. J. BABEL, *Iowa State College.*
M15. The Influence of the Quality of Milk and Curing Temperatures on the Ripening of Pasteurized-Milk Cheddar Cheese, GEORGE P. SANDERS, HOMER E. WALTER, AND RALPH P. TITTSLER, *Bureau Dairy Industry, U.S.D.A.*
M16. The Manufacture of Grating Type Cheese, GEORGE H. WATROUS, *Pennsylvania State College.*
M17. The Influence of Age and Degree of Ripeness on the Activity of Cheese Cultures, H. C. OLSON AND RUSSELL BEACHBOARD, *Oklahoma A & M College.*
M18. A Study of the Physical Changes which Effect the Storage Life of Evaporated Milk, B. H. WEBB, E. F. DEYSHER, AND C. F. HUGNAGEL, *Bureau of Dairy Industry, U.S.D.A.*
M19. Observations on the Judgment of Flavor Defects in Dairy Products, D. V. JOSEPHSON, *Ohio State University.*

3:00– 4:00 **Business Session.**
4:00– 5:00 **Committee Meetings.**
Campus Tours.

Thursday, June 20

Morning Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

9:00–11:00 **Bacteriology and Chemistry.**

M20. A Water Soluble Antioxident for Milk and Milk Products, N. P. TARASSUK AND J. L. HENDERSON, *University of California.*

M21. Isolation of Whey Proteins and their Conversion into Food Products, L. A. BURKEY AND H. E. WALTER, *Bureau of Dairy Industry, U.S.D.A.*

M22. Variations in the Acid Degree of Milk Fat as Affected by Churning and Extraction Procedures, B. C. JOHNSON AND I. A. GOULD, *University of Maryland.*

M23. Concentration of the Free Fatty Acid Portion of Milk Fat by Alcohol Extraction, I. A. GOULD, *University of Maryland.*

M24. The Direct Microscopic Count as a Method for Counting Bacteria in Pasteurized Milk, M. P. BAKER, *Iowa State College.*

M25. Non-lactose Fermenting Yeasts and Yeast-like Fungi from Cream and Butter, STANLEY H. F. CHINN AND F. E. NELSON, *Iowa State College.*

M26. Improvements in the Phosphatase Test for Milk and for Cheese, GEORGE P. SANDERS AND OSCAR S. EAGER, *Bureau of Dairy Industry, U.S.D.A.*

M27. The Relationship of the Growth of all Bacteria and Coliform Bacteria in Pasteurized Milk Held at Refrigeration Temperatures, A. C. DAHLBERG, *Cornell University.*

11:00–12:00 **Business Session**—*Room 118, Dairy Industries Building.*

Afternoon Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

1:00– 3:00 **Chemistry, Sanitation, Teaching.**

M28. Some Observations Regarding the Effect of Various Wave Lengths of Light on the Riboflavin Content and Flavor of Milk, D. V. JOSEPHSON, *Ohio State University.*

M29. Sanitization of Creamery Water Supplies with Various Cationic Bactericides, C. JENSEN, *North Dakota Agricultural College.*

M30. Manufacture of Powdered Cream Mix for Whipping by Aeration, HARRY PYENSON AND P. H. TRACY, *University of Illinois.*

M31. Influence of Nonfat Dry Milk Solids on the Nutritive Value of Bread, LLOYD K. RIGGS, ANABEL BEATY, AND ARNOLD H. JOHNSON, *Kraft Cheese Co., Chicago, Illinois.*

M32. A Device to Aid in Determining the Effectiveness of Dairy Detergents, E. I. FOUTS AND T. R. FREEMAN, *University of Florida.*

M33. Studies of a "Self Washing" Cream Separator, E. O. HERRIED, RALPH HUSSON, AND P. H. TRACY, *University of Illinois.*

M34. An "In Training" Course for Workers in the Dairy Manufacturing Industry, P. H. TRACY AND E. O. HERRIED, *University of Illinois.*

3:00– 5:00 **General Business Session,** *Agr. Assembly Room, Agr. Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

PRODUCTION PROGRAM

Tuesday, June 18

Afternoon Session

1:00– 4:00 SECTION A—**Vitamins,** *Room 19, Agr. Hall,* G. W. SALISBURY, *Chairman.*

P1. The Effect of Massive Doses of Vitamin D on the Blood Picture of Dairy Cows at Parturition, J. W. HIBBS, *Ohio Agricultural Experiment Station.*

P2. Carotene and Vitamin A in the Blood and the Liver of Newborn and Colostral Fed Calves, G. H. WISE, M. J. CALDWELL, F. W. ATKESON, AND J. S. HUGHES, *Kansas Agricultural Experiment Station.*

P3. The Placental Transmission of Vitamin A and Carotene in the Bovine, A. A. SPIELMAN, J. W. THOMAS, AND J. K. LOOSLI, *Cornell University.*

P4. Utilizing All Colostrum in Calf Feeding, H. E. KAESER AND T. S. SUTTON, *Ohio State University and the Ohio Agricultural Experiment Station.*

P5. Effect of Vitamin Supplements on Survival of Newborn Calves, R. G. HANSEN, P. H. PHILLIPS, AND I. W. RUPEL, *University of Wisconsin.*

P6. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage in Calves, J. W. HIBBS AND W. E. KRAUSS, *Ohio Agricultural Experiment Station.*

P7. The Relationship of Prepartum Diet to the Carotene and Vitamin A Content of Colostrum, J. W. THOMAS, A. A. SPIELMAN, AND K. L. TURK, *Cornell University.*

P8. Vitamin A and Globulin Content of Colostrum Milk, R. G. HANSEN, P. H. PHILLIPS, J. W. WILLIAMS, AND V. R. SMITH, *University of Wisconsin.*

P9. The Effect of Vitamin A Deficiency on Reproduction in Dairy Bulls, R. E. HODGSON, S. R. HALL, W. J. SWEETMAN, H. G. WISEMAN, AND H. T. CONVERSE, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P10. Relation of Carotene Levels to Fertility in Dairy Bulls, I. R. JONES, J. O. SCHNAUTZ AND J. R. HAAG, *Oregon State College.*

SECTION B—**Hormones, Blood, and Disease,** *Agr. Assembly Room, Agr. Hall.* D. M. SEATH, *Chairman.*

P11. The Adrenal Cortex in Relation to Ketosis in Dairy Cows, J. C. SHAW, *University of Maryland.*

P12. Effect of Stilbestrol on Lactation and Reproduction, SIDNEY MARSHALL, R. B. BECKER, AND P. T. DIX ARNOLD, *Florida Agricultural Experiment Station.*

P13. Elimination of Male Hormone by Lactating Dairy Cows, C. W. TURNER, *University of Missouri.*

P14. A Technique for Intravenous Feeding of Ruminants with Some Observations on the Effect of the Administration of Various Substances Upon the Secretion of Milk Fat, A. I. MANN, *University of Connecticut,* AND J. C. SHAW, *University of Maryland.*

P15. Blood Picture of Louisiana Dairy Cattle, L. L. RUSOFF AND P. L. PIERCY, *Louisiana State University.*

P16. Studies of the Growth and Blood Composition of Dairy Calves Fed Remade Skimmed Milk after Three Days of Age, P. M. REAVES AND L. R. ARRINGTON, *Virginia Polytechnic Institute.*

P17. The Effect of Complete Milking on Blood-Serum Calcium of Cows at Parturition, VEARL R. SMITH, *University of Wisconsin.*

P18. The Occurrence of Various Bacterial Infections and Their Significance in Bovine Mastitis, L. A. BURKEY, W. W. SWETT, AND CECELIA R. BUCKNER, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P19. Some Results in Treating Mastitis by Means of Sulfanilamide, Tyrothricin, and Penicillin, H. A. HERMAN, J. E. EDMONDSON, A. C. RAGSDALE, AND O. S. CRISLER, *University of Missouri.*

P20. The Effect of Ingestion of D.D.T. upon Dairy Cows, N. N. ALLEN, H. A. LARDY, AND H. F. WILSON, *University of Wisconsin.*

4:00– 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND G. W. SALISBURY—*Co-Chairmen*

9:00–12:00 **Joint Symposium Production and Extension Sections.**
Breeds Relations—Committee Reports.

A. Pasture and Roughage Committee, R. B BECKER, *University of Florida, Chairman.*

B. Program of the Purebred Dairy Cattle Association, G. A. BOWLING, *Secretary.*

C. Breed Relations Committee, H. A. HERMAN, *University of Missouri, Chairman.*

D. Uniform Rules for Official Testing, FLOYD JOHNSTON, *Iowa State College.*

E. Dairy Cattle Breeding Committee, C. L. BLACKMAN, *Ohio State University, Chairman.*

F. Program Suggestions for Inter-Breed Groups, E. C. SCHEIDENHELM, *Rutgers University.*

G. Round Table Discussion, BREED ASSOCIATION REPRESENTATIVES.

Afternoon Session

1:00– 3:00 SECTION A—**Thyroprotein, Protein, and Minerals,** *Room 19, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P21. Thyroid Secretion Rate of Growing, Pregnant, and Lactating Albino Rats, R. A. MONROE AND C. W. TURNER, *University of Missouri.*

P22. Some Physiological Effects of Feeding Thyroprotein to Dairy Cows, L. A. MOORE, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P23. The Influence of a Synthetic Thyroprotein When Fed to Dairy Cows Over an Extended Period of Time, RALPH P. REECE, *New Jersey Agricultural Experiment Station.*

P24. Effect of Feeding Thyroprotein to Dairy Cows on the Protein Composition and Content of Milk, A. H. VANLANDINGHAM AND GEORGE HYATT, JR., *West Virginia Agricultural Experiment Station.*

P25. The Influence of Feeding Synthetic Thyroprotein on Fertility of Bulls, A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska.*

P26. Utilization of Non-Protein Nitrogen by Dairy Heifers, I. R. JONES AND J. R. HAAG, *Oregon State College.*

P27. Shark Meal as a Protein Supplement in Dairy Calf Rations, SIDNEY P. MARSHALL, *Clemson Agricultural College.*

P28. Ground Mungbeans as a Protein Supplement in Rations for Dairy Cows, A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A & M College.*

P29. A Preliminary Report of a Study of the Effects of Manganese on Calcium Metabolism in Lactating Cows, J. T. REID, K. O. PFAU, C. B. BENDER, AND R. L. SALISBURY, *New Jersey Agricultural Experiment Station.*

SECTION B—**Feeding and Management,** *Agricultural Assembly Room, Agricultural Hall,* D. M. SEATH, *Chairman.*

P30. Needed Dairy Feed Ingredient Research, R. T. PARKHURST, *Flory Milling Company, Inc., Bangor, Pennsylvania.*

P31. Ground Ear Corn as Compared to Ground Shelled Corn in a Simple Grain Mixture for Milk Production, C. F. MONROE AND W. E. KRAUSS, *Ohio Agricultural Experiment Station.*

P32. Ten Years Evaluation of Bluegrass Pastures, H. B. MORRISON AND FORDYCE ELY, *Kentucky Agricultural Experiment Station.*

P33. Silage from Normal and from Drouth Damaged Corn, C. F. MONROE, A. E. PERKINS, C. E. KNOOP, AND LOUISE SKINNER, *Ohio Agricultural Experiment Station.*

P34. The Unknown Lactation Factors in Corn Silage, C. F. HUFFMAN, S. T. DEXTER, AND C. W. DUNCAN, *Michigan State College.*

P35. A Preliminary Report on the Study of Factors Influencing Rumen Microflora, A. L. BORTREE, K. M. DUNN, R. E. ELY, AND C. F. HUFFMAN, *Michigan State College.*

P36. The Physiological Role of the Rumen of the Young Bovine as Indicated by the Growth and Blood Composition of a Rumen-ectomized Calf, G. H. WISE, R. R. LINK, W. W. THOMPSON, AND M. J. CALDWELL, *Kansas Agricultural Experiment Station.*

P37. Effect of High Humidity and High Temperature on Dairy Cows, D. M. SEATH AND G. D. MILLER, *Louisiana Agricultural Experiment Station.*

P38. The Causes and Extent of Variations in Weight of Dairy Cows Weighed at Half-hour Intervals over Ten-day Periods, N. N. ALLEN, *University of Wisconsin.*

3:00– 4:00 **Business Session,** *Agricultural Assembly Room, Agricultural Hall.*

4:00– 5:00 **Committee Meetings.**
Campus Tours.

Thursday, June 20

Morning Session, *Agr. Assembly, Agr. Hall*

FLOYD J. ARNOLD AND G. W. SALISBURY—*Co-Chairmen*

9:00–11:00 **Joint Symposium Production and Extension Sections.** Herd Health and Sanitation—a panel discussion led by W. E. PETERSEN, *University of Minnesota.*

11:00–12:00 **Business Session**—*Agricultural Assembly Room, Agricultural Hall.*

Afternoon Session

1:00– 3:00 SECTION A—**Hay,** *Room 19, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P39. Standardizing Hay Palatability Trials with Dairy Calves, H. S. WILLARD, *University of Wyoming.*

P40. Comparative Value of Alfalfa, Mixed Grasses and Legume, and Timothy Hays for Young Dairy Calves, C. L. NORTON, *Cornell University.*

P41. Feeding Barn Dried Hay to Dairy Heifers, C. E. WYLIE, JOHN EWING, AND S. A. HINTON, *University of Tennessee.*

P42. Progress Report on Comparing the Efficiency of Three Methods of Harvesting and Preserving Forage Crops, R. E. HODGSON, J. B. SHEPHERD, L. G. SCHOENLEBER, H. M. TYSDAL, AND W. H. HOSTERMAN, *Bureau of Dairy Industry, Bureau of Plant Industry, Soils and Agricultural Engineering, and Production and Marketing Administration, Agricultural Research Administration, U.S.D.A., cooperating.*

P43. Nutritive Value of Barn-cured Hay, K. L. TURK AND C. L. NORTON, *Cornell University.*

P44. A Still Unidentified Nutrient in Roughages and in Milk, A. M. HARTMAN AND C. A. CARY, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P45. Fan Ventilated Long Hay: A Chemical Study, A. E. PERKINS AND LOUISE SKINNER, *Ohio Agricultural Experiment Station.*

P46. Comparative Feeding Value of Wilted Alfalfa Silage and Alfalfa Hay for Dairy Cows, J. B. SHEPHERD AND W. J. SWEETMAN, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P47. Early vs. Late Cut Lespedeza Hay for Dairy Cows, JOHN EWING AND C. E. WYLIE, *University of Tennessee.*

SECTION B—**Breeding,** *Agricultural Assembly Room, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P48. Crossing Zebu with Northern Breeds of Cattle in Jamaica, JOHN W. HOWE, *Iowa State College.*

P49. Some Experiences with Central Artificial Breeding Organizations, H. A. HERMAN, W. K. DINWIDDIE, AND M. J. REGAN, *University of Missouri.*

P50. The Use of Pregnancy Diagnosis with Artificial Breeding, L. E. CASIDA, G. R. BARRETT, AND C. A. LLOYD, *University of Wisconsin.*

P51. The Effect of Certain Coal-Tar Dyes Used for Semen Identification on the Livability and Fertility of Bull Spermatozoa, J. O. ALMQUIST, *Pennsylvania State College.*

P52. The Effect of Feeding Potassium Iodide and Skim Milk Powder on Spermatogenesis, C. E. KNOOP, *Ohio Agricultural Experiment Station.*

P53. Seasonal Effect on Spermatogenic Activity in the Bull, ERNEST MERCIER, *Cornell University and Ministry of Agriculture of Quebec Province, Canada.*

P54. Time of Insemination and Conception Rate in Artificial Breeding, G. R. BARRETT AND L. E. CASIDA, *University of Wisconsin.*

P55. Artificial Insemination as a Means of Genetic Improvement, J. L. LUSH, *Iowa State College.*

P56. Deleterious Recessive Genes in Dairy Bulls Selected at Random, S. W. MEAD, P. W. GREGORY, AND W. M. REGAN, *University of California.*

3:00– 5:00 **General Business Session,** *Agricultural Assembly Room, Agricultural Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

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THE INFLUENCE OF AGE ON BREEDING EFFICIENCY OF DAIRY CATTLE IN ARTIFICIAL INSEMINATION

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Age, undoubtedly, exerts an influence on breeding efficiency of dairy cattle. Little agreement on the effect of age on fertility exists beyond the general conclusions that the number of services required per conception by heifers is significantly greater than for cows that have calved once or more (2, 3, 8, 10, 11, 13, 14, 15, 20, 21). Also, for cows older than nine or ten years there appears to be a considerable increase in the number of services required per conception (10, 11, 15, 16).

Conflicting results have been reported by others (1, 12), who noted little difference in the number of services required for conception by cows of various ages, including the two-year-old group. Gowen and Dove (9) found a trend of gradual reduction in breeding efficiency with advancing age, starting with heifers under one year of age. Lasley and Bogart (13) in an artificial-breeding project with beef cattle found that the peak of fertility occurred with cows five and six years old, followed by a gradual decline with advancing age.

Starting with yearling bulls which have, in general, the highest fertility index, a gradual, but definite, decline in breeding efficiency of bulls accompanies advancing age when measured by the number of services required per conception (2, 3, 7, 9, 10, 15). Dawson (5) observed a similar declining trend in breeding efficiency of aged bulls between the ages of five and sixteen years.

On the other hand, the New Zealand Dairy Board (16) found that up to ten years of age no significant correlation between age and fertility was evident. Others have found a higher breeding efficiency resulting from the use of young bulls under six years of age, as compared with older bulls, when bred to either cows of all ages (10, 14) or to first-gestation heifers (10, 15).

Although the growing popularity of artificial insemination has greatly

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increased the breeding potentialities of many bulls, little attention has been devoted to the question of age of the mates as a factor in determining the efficiency of the practice.

This study has been undertaken to determine the effect of age of both dam and sire upon the breeding efficiency of dairy cattle in artificial breeding in which there is a preponderance of aged bulls in use. Also, it is now possible under conditions of artificial insemination to determine whether or not reported low fertility of young heifers in natural service is due to physical incompatibility of the mates, or to an inherent lower fertility of young heifers.

EXPERIMENTAL

The data included in this investigation consist of services only to registered Holstein-Friesian cows bred by artificial insemination for which service records were complete and known to be accurate. All grade cows have been sifted out, although complete breeding histories were available, since in most commercial herds it has not been possible to obtain satisfactory records of their date of birth. As a consequence, too frequently their ages have been mere approximations rendering yearly classifications highly inaccurate.

All the services included in this study have been taken from the breeding records of the New York Artificial Breeders' Cooperative from the beginning of the association in June, 1940, until June 30, 1944. Over this period of four years a total of 12,621 services to purebred Holstein-Friesian heifers and cows became available. As summarized in table 1, of this number 8,350 were first services, or services to cows bred for the first time for any single gestation, and 4,271 were second services, or services to cows which had failed to conceive from the first insemination. The number of services to purebred cows represented 22.4 per cent of the total services to the Holstein-Friesian bulls in the period covered.

To determine the age of each cow and bull the date of birth was traced back into the Holstein-Friesian Herd Books through their registration numbers. All cows were grouped in full-year periods, as seen in table 1. From year to year many cows have reappeared, their re-entry depending upon the age at which they were again bred for a later calf. The average age of the 8,350 cows was 4.2 years.

The data were further tabulated and arranged to disclose the breeding efficiency of the 41 bulls during the time they remained in service for the Cooperative. The ages of the bulls ranged from one to twelve years.

The innate fertility of all these bulls, compared with the cows to which they were mated, may be more accurately assessed, since contributory factors of environment and management have been similarized as much as possible for all bulls. On the average, semen samples were drawn from each bull once every seven to ten days. This uniformity in the frequency of collection would tend to minimize the effect noted by Dawson (5) that conception rate in natural service is an inverse function of the number of previous monthly services.

No discrimination could be made between sterile and fertile cows; therefore, all services to cows, fertile or sterile, were credited or discredited to the respective bulls. Nor was it possible to analyze the data beyond first and second services, since the success of most third services is unknown. Under the present adopted policy, a cow failing to conceive on the first service is entitled to two additional services for the original service charge. Beyond the third service an extra fee is charged. For this reason. after the third service some breeders have resorted to natural breeding or to the disposal of cows which have failed to conceive. Due to this fact there is no reliable way to determine the outcome of the third service. Therefore, third services have been used only as an indication of the success or failure of second services.

When recording the data, the assumption was made that all cows showing no recurrence of estrus during the succeeding five-month period were pregnant unless definitely stated to the contrary. This five-month interval after insemination has been chosen to signify conception since Elliott (6), in studying the results of one local association, found that 95 to 99 per cent of the cows inseminated but returning to service thereafter had already returned during the five months following the last service. Thus, all data in reference to conception rate are based upon the per cent of non-returns during a period of five months after service.

It is recognized that there may be certain confounding factors operative in this study to influence the absolute accuracy of the conception rates reported here. Apparently purebred breeders are somewhat more tolerant of cattle which fail to conceive readily than owners of grade cattle for the actual conception rate for the purebreds considered is about 3 per cent lower than for all cows bred to the Holstein-Friesian bulls during the same period of time. Also, the true effect of age on conception rate may not be shown in these studies for females of the younger ages which failed to conceive were constantly being culled and would not appear in the older age groups. However, it is believed that the sample of cows studied is representative of the cow population in New York and affords an opportunity for establishing a broad generalization regarding their breeding efficiency.

RESULTS

Over-all breeding efficiency. The over-all percentage of conceptions was found to be 48.2 per cent, or equivalent to 2.07 services per conception. In other words, out of 12,621 services to purebred Holstein-Friesian cows 6,538 inseminations were fruitless. This figure of 2.07 services per conception is a reflection of not only the inclusion of barren cows and heifers but of problems peculiar to artificial insemination. These have included the shipment of semen by parcel post and its storage at terminal points until used.

Salisbury *et al.* (17, 18) found that less than 1 per cent to probably no more than 4 per cent of all services during the period covered by this study were made on the day of semen collection.

Another factor that has contributed to the low conception rate of 48.2 per cent comes from inefficiency encountered during the experimental stage of development of each new member-association. This study covers a period of rapid growth during which time the number of member-associations of the New York Artificial Breeders' Cooperative, Inc., increased from 5 to 26.

Influence of age of cow on breeding efficiency. The average percentage of conception, as shown in table 1 was calculated for each age group of cows when bred to bulls of all ages.

TABLE 1

Influence of age of cow on conception rate

Age of cow (years)	First services		Second services		Total services	
	No.	% non-returns	No.	% non-returns	No.	% non-returns
1	1022	44.6	504	38.7	1526	42.7
2	1324	47.4	678	45.1	2002	46.6
3	1478	49.4	742	48.2	2220	49.0
4	1226	53.4	591	47.9	1817	51.6
5	989	49.4	502	49.0	1491	49.3
6	825	50.3	419	51.8	1244	50.8
7	544	48.2	306	51.3	850	49.3
8	405	46.4	217	47.9	622	46.9
9	262	45.4	150	48.7	412	46.6
10	140	49.3	79	53.2	219	50.7
11	89	40.4	54	42.6	143	41.3
12 & over	46	43.5	29	44.8	75	44.0
Totals and percentages	8350	48.69	4271	47.23	12,621	48.20

These summarized figures conclusively establish the fact that in artificial insemination yearling heifers, as a group, require more services per conception than cows of subsequent age groups up through ten years.

It is interesting to compare the curve obtained from first services with that arising from plotting the points of second services. Up to five years of age, the second service conception curve parallels that resulting from first services except at a lower level. Then at this point a complete reversal of the levels of the two curves occurs. Though still running parallel, the second service conception curve now maintains the higher level. In other words, cows under five years of age have a higher conception rate on first service than on second, while in cows over five years of age the percentage of cows conceiving on second service exceeds that from the first.

The results of this study again emphasize a fact observed in other investigations but not always receiving the attention it merits. That is, reproductive functions reach their maximum efficiency after the second or third gestation, maintain this level for a few years, then begin to decline with advancing age. With the exception of the steep ascent of the ten-year-old group, due to small numbers, a fairly uniform and intelligible breeding efficiency curve is portrayed by plotting the figures in table 1.

Influence of age of sire on breeding efficiency. To study the influence of the age of the sire on breeding efficiency, the rate of conception for bulls of each age was calculated upon services to cows of all ages, as shown in table 2. It is readily seen that in artificial insemination the influence of age of the sire is given no opportunity of following its normal course, since bulls are so rigidly selected and culled for fertility. For this reason the apparent effect of age of the bull in the determination of breeding efficiency is much less marked than the age of the cow, irrespective of this method of grouping, whether by first, second, or total services. Thus, the forms of the two curves obtained by plotting the data in tables 1 and 2 are noticeably different.

TABLE 2

Influence of age of sire on conception rate

Age of bull (years)	First services		Second services		Total services	
	No.	% non-returns	No.	% non-returns	No.	% non-returns
1	222	53.6	115	50.4	337	51.6
2	539	58.1	309	52.8	848	56.1
3	836	50.6	415	48.7	1251	50.0
4	369	49.3	200	46.0	569	48.2
5	456	42.1	263	51.7	719	45.6
6	1009	42.2	515	41.4	1524	41.9
7	1413	49.9	674	46.4	2087	48.8
8	818	51.6	464	48.7	1282	50.5
9	946	48.3	511	45.4	1457	47.3
10	1140	48.4	510	47.8	1650	48.2
11	530	46.0	246	45.9	776	46.0
12	72	43.1	49	57.1	121	48.8
Totals and percentages	8350	48.69	4271	47.23	12,621	48.20

Nevertheless, the points of the age curve for bulls are striking and at variance with the general trend of breeding efficiency found to exist in natural breeding where, starting with yearling bulls, a gradual decline in fertility accompanies advancing age. In this study the only outstanding high figure in the uniform level of breeding efficiency of bulls of varying ages occurs in the two-year-old group: 56.1 per cent, as compared with the average of 48.2 per cent for bulls of all age groups combined. The yearling group, though above the average conception rate, failed to surpass the high level of conceptions of the two-year-old group.

Undoubtedly, the rapid fall in conception rates involving the five-year-old and six-year-old bulls is due to the large number of proved bulls that enter the Cooperative at this age and to the subsequent rigorous culling process which resulted in the bulls in the next age groups being higher than average in fertility.

The data in table 2 may be interpreted as representing a bimodal curve, describing two populations of bulls. The trend of the first four points represents young bulls going into maximum breeding efficiency as two year olds, then waning in fertility with age. The remainder of the curve represents proven bulls which enter the Cooperative at the age of five and six years, unculled for fertility level. Following the disposal of bulls of low fertility the eight-year-old bulls remain a highly selected group which gradually declined in fertility with advancing age.

Influence of age of both dam and sire on breeding efficiency. In this study of 41 bulls the rate of conception varied from 26 to 61 per cent, emphasizing fully the prominent rôle played by the individual bull in determining breeding efficiency. Within any age group, individual variations among bulls have been, in some cases, so great that they have completely overshadowed the difference that might have been due to age.

Out of this study has come no simple regression of conception rate on age of either bulls or cows. It is obvious that the relationship between the age of cows, or bulls, and conception rate in artificial insemination is not a simple linear regression.

At the present time insufficient data on bulls are available to determine the true relationship of age to fertility. It would be desirable to have data on a relatively large number of bulls, all used for several years. Even this might not show the true relationship, for such data would be highly selective and exclusive. That is, continued service in artificial insemination for such long periods would invariably lead to the retention of only bulls of relatively high fertility.

To determine the statistical significance of these data an analysis of covariance (Snedecor, 1940), using both first and second services, was made. In order to eliminate the effect of the number of services upon the efficiency of conception, the number of services for each age of bull and each age of cow was taken as the independent variable, x, and the number of five-month non-returns to service for each of these criteria of classification as the dependent variable, y. From this analysis a highly significant difference between the various ages of both cows and bulls was found; the odds in both cases were greater than 99 : 1 that the effect was not due to chance alone.

It is interesting to note in this connection that an analysis of the unweighted percentages of conception showed no significant differences between ages of cows or ages of bulls. The problem of the analysis of percentages for such data has been discussed by Salisbury *et al.* (17), and by Cochran (4).

The data were not amenable to statistical determination of the interaction between the various ages of bulls and ages of cows. However, when cows and bulls are classified into three-year age groups, a possible interaction seems noticeable. A careful study of the percentages of conception in table 3 indicates that young bulls between the ages of one to three and cows between the ages of four and six possess a higher breeding efficiency than any other respective group. The highest rate of conception, 54.0 per cent, resulted from the mating of cows and bulls of these two groups.

On the whole the influence of the age of the cow and the age of the sire on breeding efficiency in artificial insemination is very similar to that reported for natural breeding. The lower rate of conception reported for uncalved heifers in natural service has been found equally applicable under conditions of artificial insemination where the incompatability of size and weight is no longer an influencing factor. The characteristic decrease in the level of fertility of cows 10 years of age and over also holds true in artificial insemination. Despite the attempt to retain and use highly fertile bulls in artificial insemination, young bulls between the ages of one and three years, maintain highest levels of fertility.

TABLE 3

Influence of age of both dam and sire on conception rate

Age of bull (years)		Age of cows (years)				
		1–3	4–6	7–9	10 & over	All ages
1–3	%	52.1	54.0	51.0	43.1	52.3
	No.	1157	848	359	72	2436
4–6	%	40.9	46.9	46.4	48.1	44.1
	No.	1285	988	435	104	2812
7–9	%	46.7	52.2	46.1	51.1	48.8
	No.	2179	1775	709	163	4826
10–12	%	46.7	48.6	50.1	38.8	47.6
	No.	1127	941	381	98	2547
All Ages	%	46.5	50.6	47.9	46.5	48.2
	No.	5748	4552	1884	437	12621

SUMMARY

1. Since the beginning of the New York Artificial Breeders' Cooperative in June, 1940, until June 30, 1944, a total of 12,621 complete, recorded services to registered Holstein-Friesian cows, involving 41 bulls, have accumulated. This number is 22.4 per cent of all matings to Holstein-Friesian bulls.
2. The average number of services required per conception when based on all females, infertile cows included, was 2.07, or a breeding efficiency of 48.2 per cent.
3. The influence of the age of the cow on breeding efficiency reveals a steady increase in the conception rate up to four years of age. Between the ages of five to seven years, inclusive, cows maintain a uniformly high breeding efficiency, which gradually declines with advancing age.
4. Although the full effect of the age of the sire could not be obtained with a group of bulls selected for fertility, young bulls between the ages of one to three years, inclusive, have shown the highest breeding efficiency of all age groups. Within this group the peak in conception rates was found for the two-year-old bulls.

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VARIATION BETWEEN BREEDS IN FEED COST OF MILK PRODUCTION

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INTRODUCTION

In 1943 a program of herd analyses was undertaken by the Dairy Extension Department of the University of Kentucky for all herds finishing a year's work of D.H.I.A. testing. Included in the analysis data sent to each owner are the feed cost figures for producing one pound of butterfat and 100 pounds of milk. Routine examination of these herd-analysis charts seemed to rather consistently show breed differences in the feed cost of producing one pound of butterfat and 100 pounds of milk.

For years, the American Jersey Cattle Club has claimed that one of the attributes of the Jersey breed is economy of butterfat production. Also the Holstein-Friesian Association has advertised the Holstein cow as a low-cost milk producer. Since most of the herds doing D.H.I.A. testing in Kentucky are composed of these two breeds, some interesting comparisons were possible.

PROCEDURE

Feed costs vary over Kentucky, but taking two full associations in the Louisville area for 1945 it was found that eighteen herds composed of either all registered Jerseys, or part registered and part grade Jerseys produced butterfat at a feed cost of $0.34 a pound. Twenty herds of either all registered or part registered and part grade Holsteins in the same two associations produced butterfat with a feed cost of $0.41 a pound. On the other hand, the eighteen Jersey herds had a feed cost of $1.75 for producing 100 pounds of milk, while the twenty Holstein herds produced 100 pounds of milk with a feed cost of $1.49. These results, in herds, all tested in the same three counties, where feed costs and pasture conditions are similar, tend to substantiate the respective claims of the two Breed Associations.

In 1928, Gaines (1) published the final results of his studies showing that the energy yield, that is the gross energy value of the milk, is a better measure of yield than either milk or fat alone. The formula which he published is now well known, but for illustration it is:

$$E' = 0.4M + 15F$$

Where E' is energy value in terms of 4 per cent fat-corrected milk in pounds, M is milk yield in pounds and F is fat yield in pounds. One pound 4 per

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TABLE 1

Kentucky D.H.I.A. cost of production data for the years 1943, 1944, 1945

Breed	Average feed cost per 100 lbs. 4% fat-corrected milk
28—All registered Holstein herds	$1.34
76—All registered Jersey herds	$1.36
41—Part registered and part grade and all grade Holstein herds	$1.50
46—Part registered and part grade and all grade Jersey herds	$1.46
115—All registered herds, including all breeds	$1.35
48—Part registered and part grade herds	$1.47
110—All grade herds	$1.51

cent fat-corrected milk = 340 kilocalories of milk energy. Expressing milk yield in terms of energy value, that is in terms of 4 per cent fat-corrected milk, is a much more reliable measure than is either milk yield or fat yield alone.

The herd analysis chart prepared for each owner shows the total milk yield of the herd, the total fat yield of the herd and the total feed cost for the D.H.I.A. testing year for 365 days. These yields for all herds finishing a year's work during the years 1943 to 1945 inclusive were computed to a 4 per cent F.C.M. basis using the foregoing formula. Then the feed costs of producing 100 pounds of 4 per cent F.C. milk was determined for each herd. The results are shown in table 1.

The Kentucky Agricultural Experiment Station herd is composed of two breeds, registered Jerseys and registered Holsteins. The cattle are all fed the same ration (except for amounts) and handled alike. This gave two breeds fed under exactly the same conditions. The feed cost per pound of butterfat, per 100 pounds of milk, and per 100 pounds of 4 per cent F.C.M. are given in table 2.

As previously stated, feed costs vary over the state. It is recognized that the cheapest milk is made on grass, and the grazing season is longer in the southern part of the state. To eliminate any climatic or geographic errors, all the herds finishing a year's work in 1945 in two full associations operating in the Louisville Milk Shed Area were studied in detail. A few

TABLE 2

Kentucky Agricultural Experiment Station dairy herds (cost of production data)

	Year	Reg. Jerseys	Reg. Holsteins
Average feed cost per 1 lb. butterfat	1944	$0.38	$0.44
Av. feed cost per 100 lbs. milk	1944	$2.00	$1.59
Av. feed cost per 100 lbs. 4% F.C.M.	1944	$1.68	$1.69
Av. feed cost per 1 lb. butterfat	1945	$0.34	$0.39
Av. feed cost per 100 lbs. milk	1945	$1.84	$1.53
Av. feed cost per 100 lbs. 4% F.C.M.	1945	$1.53	$1.55

herds of Guernseys, and a few of mixed breeding were discarded. Only those herds of either all registered Holsteins or part registered and part grade Holsteins, and all registered Jereys, or part registered and part grade Jerseys were used. Table 3 shows the results of this tabulation.

TABLE 3

1945 D.H.I.A. Shelby-Boone-Carroll and Oldham Jefferson Associations	Reg. or reg. and grade Jerseys	Reg. or reg. and grade Holsteins
No. of herds	18	20
Av. fat yield per cow	382.4 lbs.	378.4 lbs.
Av. milk yield per cow	7373 lbs.	10425 lbs.
Av. fat percentage	5.19 %	3.63 %
Av. value of product per cow	$314	$363
Av. feed cost per cow	$129	$150
Av. value of product above feed cost per cow	$185	$213
Av. feed cost per 1 lb. butterfat	$0.34	$0.41
Av. feed cost per 100 lbs. milk	$1.75	$1.49
Av. feed cost per 100 lbs. 4% F.C.M.	$1.49	$1.52
Av. return per $1.00 spent for feed	$2.43	$2.37

DISCUSSION

In all of these tables it was observed that Jerseys consistently showed the lowest feed cost per pound of butterfat, while Holsteins produced 100 pounds of milk with the lowest feed cost. However, on a basis of energy value, as expressed in 4 per cent F.C.M. there was little difference between the respective breeds in feed cost. In the first table, the Holstein breed showed a very slightly lower feed cost per 100 pounds 4 per cent F.C.M. Yet even this small difference might be explained by the fact that the Jersey herds were charged the same amount for pasture, per cow, per month, as were the Holsteins. It is doubtful that a 1,000-pound Jersey on pasture will consume quite as much grass as will, for instance, a 1,500-pound Holstein. The usual charge was $2.00 per cow per month for pasture for a grazing season of from six to seven months.

The last part of table 1 is rather significant, in that it indicates that there is a difference in feed costs between registered herds, and herds of all grade cattle. The feed cost per 100 pounds of 4 per cent F.C.M. was noticeably lower in the purebred herds than in the herds of grade cows. How much of this difference is due to the cows alone and how much to management and feeding is a question. Undoubtedly, the purebred herds were better managed and better fed than the grade herds.

SUMMARY

1. Jersey cows produced butterfat at a lower feed cost per pound than Holsteins.

2. Holstein cows produced milk at a lower feed cost per 100 pounds than Jerseys.

3. On the basis of energy as expressed in 4 per cent F.C.M., there appeared to be no significant difference between breeds in cost of production.

4. Purebred herds apparently produced 4 per cent F.C.M. at a lower feed cost than did grade herds.

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THE EFFECT OF FEEDING COD-LIVER OIL ON THE "OXIDIZED" AND "GOATY" FLAVORS AND VITAMIN C IN MILK

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The purpose of this study was to determine the effect of feeding cod-liver oil to cows on the flavor of their milk. It was soon found that the "oxidized" and "goaty" (5) flavors were the only ones of significance, although the flavor of the oil itself was a factor at a certain point of the experiment.

The so-called "oxidized" flavors (1) include "papery," "cardboardy," "oily," "metallic," "fishy," and "tallowy." The "goaty" flavor, likewise, has a name for what it resembles. It is more specific in nature than some of the oxidized flavors. Its origin is caprylic (2) acid.

Inasmuch as vitamin C (4, 6) analyses could be made readily with other studies on ascorbic acid in our laboratories, data were obtained on the vitamin C content of all the samples of milk that were examined in this particular research on flavors. Two experiments involving 10 cows were employed in obtaining the data. The effect of feeding cod-liver oil to the cows in this study was variable. There was an indication in the first experiment that a correlation existed between the feeding of cod-liver oil and the amount of vitamin C in the milk. A second study, therefore, was made which extended over a longer period and in which more cows were included.

FIRST EXPERIMENT (1938–39)

The first experiment involved 4 cows that were in a study which already was under way in the Department of Animal Husbandry. Because of the nature of this study no planned rest periods were provided during the course of the experiment, however a rest from cod-liver-oil feeding of 1 day for cow No. 1 and of 16 days for cow No. 3 was provided.

The grain ration that was fed was composed of the following items: 200 pounds of linseed-oil meal, 400 pounds wheat bran, 400 pounds hominy and corn meal, 370 pounds heavy grain oats, 300 pounds corn distiller's dried grains, 300 pounds cocoanut-oil meal, 20 pounds steamed bone meal, and 10 pounds salt. The grain was fed to these cows according to their milk production. These animals, also, received about 1 pound of timothy hay and 3 pounds of corn silage per 100 pounds body weight. The cod-liver oil was a commercial product sold for animal feeding and was fed in quantities that are mentioned later.

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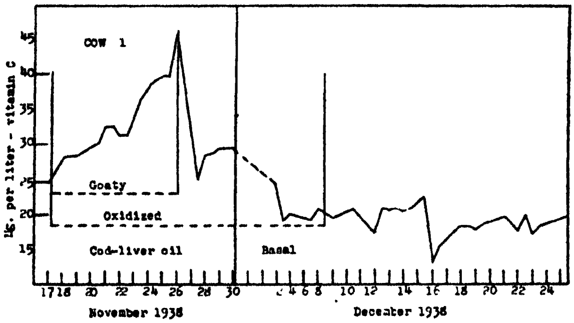

FIG. 1

The oil was administered by drench to cow No. 1 from November 16 to 30 at the rate of 400 ml. once a day. This was at the rate of 0.75 ml. per kilogram weight of cow. Figure 1 shows the record of this cow from November 16 to December 25, 1938. The vitamin C of the fresh milk increased rapidly from 24.85 to 46.38 mg. per liter. At this point the cow was seized by convulsions. She was given a rest of one day and afterwards the dose of cod-liver oil was slightly decreased for the remaining three days of the experiment. The "oxidized" and "goaty" flavors appeared immediately on the rise of the vitamin C. The "goaty" flavor did not appear in the milk after the vitamin C peak was reached. The oxidized flavor, on the other hand, continued to develop in the milk for 12 days after the high point of vitamin C was reached. It, also, should be pointed out that the oxidized flavor did not develop in the milk during the remainder of the basal period. The daily production of milk took a decided drop when the vitamin C content was high, and the milkfat test also decreased from 3.7 to 2.5 per cent.

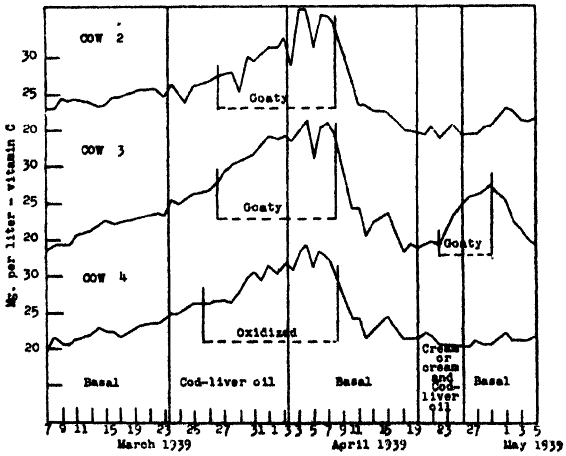

FIG. 2

The data for cows 2, 3, and 4 in this experiment are presented graphically in figure 2. It should be observed that the "goaty" flavor appeared in the milk of cows 2 and 3, two days after the "oxidixed" flavor was recognized in the milk of cow 4, and that both flavors ceased immediately after the peak in vitamin C was reached. At the end of a rest of 16 days, cow 3 was again drenched with cod-liver oil, at the rate of 0.75 ml. per kilogram of body weight. Again the "goaty" flavor appeared on the upward trend of the vitamin C. This time cow 3 was drenched daily for only one week. The other cows were not given cod-liver oil and, as expected, they did not respond with increased vitamin C nor with the production of the "goaty" and "oxidized" flavors.

SECOND EXPERIMENT (1939–40)

The concentrate mixture that was fed in this experiment was made up as follows: 220 pounds of 41 per cent protein soybean-oil meal, 240 pounds 34 per cent protein old process linseed meal, 200 pounds wheat bran, 370 pounds hominy feed and corn meal, 300 pounds ground oats, 300 pounds corn distiller's dried grains, 240 pounds 20 per cent protein cocoanut-oil meal, 100 pounds cane molasses, 20 pounds steamed bone meal, 10 pounds salt. The amount of grain that was fed to each cow depended on the amount of milk that she produced. Each cow received 1 pound hay and 3 pounds silage per 100 pounds body weight. These cows also received dry beet pulp, cottonseed meal, cream, molasses syrup, and cod-liver oil in various proportions.

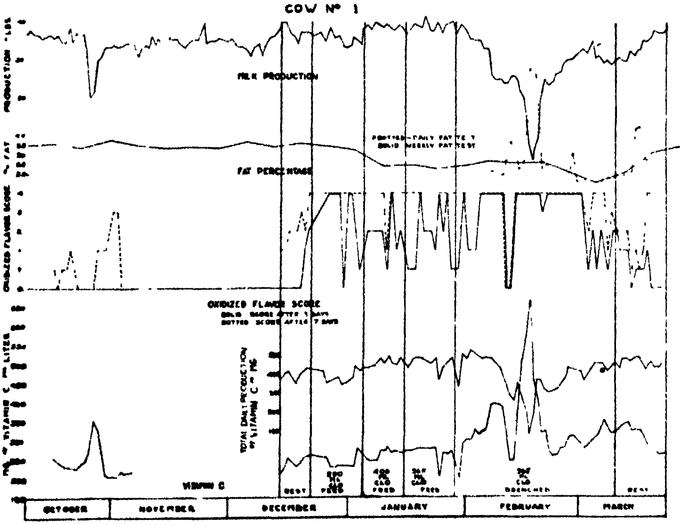

FIG. 3

It was thought that the feeding of the cod-liver oil by mixing it in the feed rather than by drenching would be more satisfactory in handling the animals and in the functioning of the nervous systems of the cows. The procedure of application was to thoroughly mix it with dried beet pulp. Usually water was added to make it more palatable. The results are shown graphically in figures 3, 4, 5, 6, 7, and 8.

Cows 1 and 2 were drenched a part of the time, when it was found that feeding by mixing with the feed did not give results. The other 4 cows in this experiment received their cod-liver oil only in the feed.

Cow 1 was fed cod-liver oil between October 17 and November 6, when she was dropped from the experiment. On December 22 she was put back for two reasons. First, she was one of the least valuable cows in the herd; and second, she was the cow in the first set that responded most readily when drenched with cod-liver oil. From December 22 to January 5 she received 0.5 ml. cod-liver oil per kilogram body weight. On that date the cod-liver oil was increased to 0.75 ml. per kilogram body weight. Then on January 15 the cod-liver oil was increased to 1 ml. per kilogram body weight and the feeding continued to January 28. At this time the same amount of cod-liver oil was administered by drenching, which was kept up until March 10. The experiment terminated by a rest period that lasted from March 10 to 22.

The drop in the milk production starting with October 26, the rise in vitamin C, and the increase in the oxidized flavor as shown in figure 3 are unexplainable. The most important features of these graphs are the abrupt drops in milk flow and in total production of vitamin C, and the equally abrupt rises in milkfat content of the milk and the vitamin C content of the milk. The oxidized flavors were increased from the mild stage to a maximum in only a few days of feeding, although there was a distinct variation from day to day in the milk that had been stored 7 days. When the cow was drenched from January 28 to March 10, the oxidized flavors were at their worst and then during the final rest period these flavors became less intense. Mention should be made that the fresh milk showed a slightly oily flavor when cow 1 was drenched with the high feeding level of cod-liver oil (1 ml. of oil per kilogram body weight). Only for a period of a few days did this oily flavor appear distinctly as cod-liver oil, and that was immediately after the drop from the peak days of February 16, 17, and 18.

The milk production of cow 1 ranged from 41.5 pounds on January 21 down to 3.3 pounds on February 17. It should be observed that the first decided drop occurred on October 27 when she gave 20.1 pounds of milk. Apparently these two restrictions in milk flow were caused by drenching with cod-liver oil. The solid line showing the weekly-fat-test curve in figure 3 was drawn from the weekly analyses recorded by the Department of Animal Husbandry. The samples were taken on a certain day each week and were not composites for a week. This explanation accounts for the weekly fat test being graphed as 2.6 per cent during the week when on one day the flow of milk dropped to 3.3 pounds and when the fat test of that milk actually was up to 27.5 per cent.

The effect of the administration of the large amount of cod-liver oil to cow 1 was interesting and the way she resisted the shock was astonishing. When she reached the peak of vitamin C, she had a convulsion. She was given one day of rest and then the same amount of cod-liver oil was fed as before, by drenching. Her resistance was shown by the way she pulled back in milk flow, total production of vitamin C, and the daily test of vitamin C. The percentage of milkfat, however, continued to drop until it was down to 1.4 per cent, having gone down from a peak of 27.5 per cent and an average of 3.0 per cent.

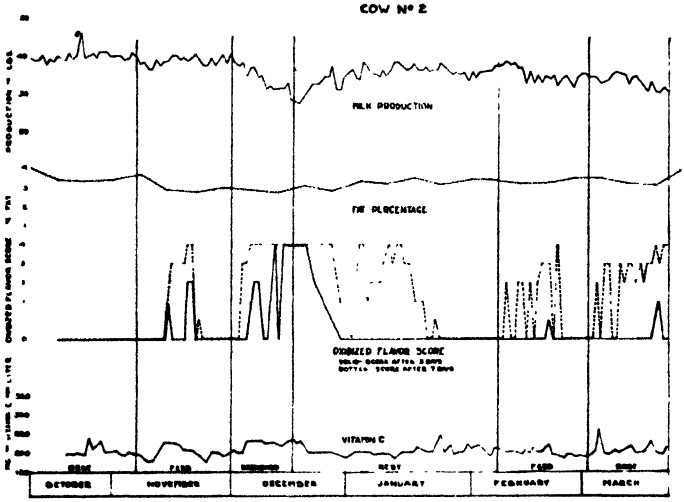

FIG. 4

Cow 2 (figure 4) produced milk that did not become oxidized on either the third or seventh days during the first rest period. When she was fed cod-liver oil from November 6 to 30 at the rate of 0.5 ml. per kilogram body weight, the oxidized flavor appeared and then disappeared. Drenching from November 30 to December 16 at the same rate and then to 0.7 ml. per kilogram body weight until December 16, distinctly increased the oxidized flavor, which disappeared during the rest period from December 16 to February 8. These flavors again appeared during the feeding of cod-liver oil at the rate of 0.5 ml. per kilogram body weight from February 8 to March 2, and remained to the end of the final rest period. There was a short interval of no oxidized flavor, however, extending over the latter part of the last feeding period to a point well in the last rest period. The vitamin C content of the milk remained unchanged, the milkfat test dropped slightly during the cod-liver oil intake periods, and the milk production dropped distinctly at the end of the drenching period.

Cow 3 (figure 5) produced milk that did not become oxidized with the exception of one instance when a question was raised on the sample after

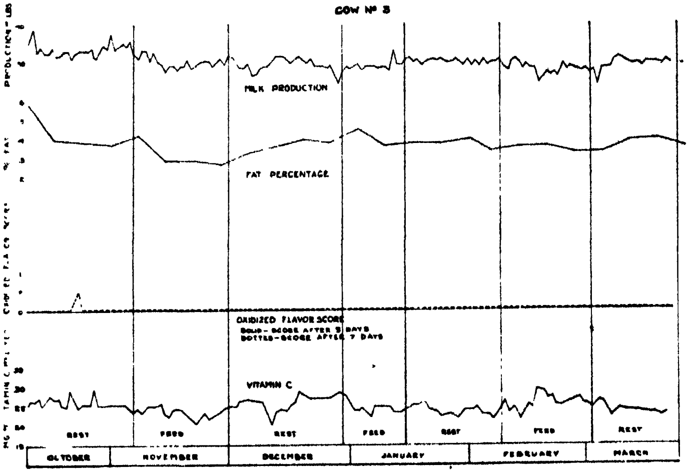

FIG. 5

7-day storage. The percentage of milkfat fell to 2.9 per cent during the first feeding period and then returned to normal. Seemingly this cow had strong resistance, for on all four items in the figure there was little variation.

Cow 4 (figure 6) gave milk that developed the oxidized flavor on 4 days on the 7-day examination. It did not appear on the third day. The percentage of milkfat dropped to the lowest point during the latter part of the first feeding period. The "goaty" flavor appeared at this time. It lasted from November 24 to December 4. This was the only time that the "goaty" flavor appeared in the second experiment. It should be noted that the vitamin C went up when the milk flow dropped on February 24.

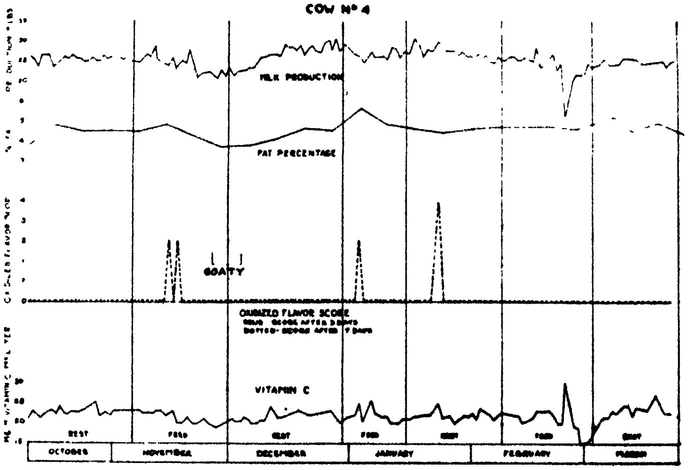

FIG. 6

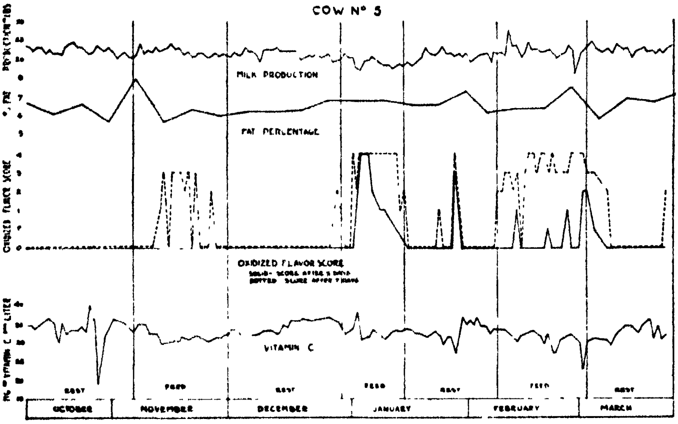

FIG. 7

Cow 5 (figure 7) shows a record of increase in the oxidized flavor during the feeding periods. No explanation is made for the drop in vitamin C on October 28 and March 1.

Cow 6 (figure 8) shows the oxidized flavor in the first rest period. During the other rest periods practically no oxidized flavor was observed, whereas during the codliver oil feeding periods, the oxidized flavor was frequently observed, particularly in the samples stored for seven days.

DISCUSSION OF VITAMIN C RESULTS

Inasmuch as some of the samples, as shown in figures 1 and 3, were extremely high in vitamin C, the question naturally arose as to whether the substance that was titrated as reduced ascorbic acid was all or only in part

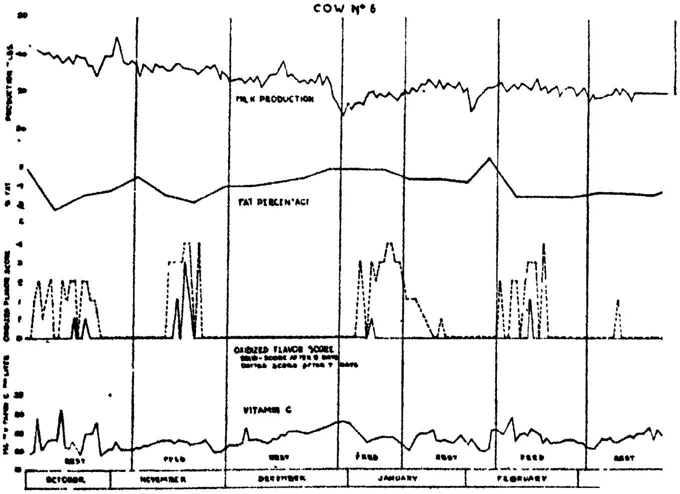

FIG. 8

reduced ascorbic acid. In an attempt to answer this question 23 samples of the milk from cow 1 on February 19 and 20, 1940, were deaerated, placed in ½-pint milk bottles, covered with mineral oil and stored at 0–10° F. The plan was to arrange a guinea-pig-feeding assay for the identification of vitamin C. A more simple and easier means of assay, however, presented itself within a year.

After these 23 samples of milk had been stored for 2 years, specific chemical tests for the identification of ascorbic acid in milk were applied to them. The samples were melted at room temperature during a period of about a day. A 50-ml. portion of the milk then was drawn by pipette from each sample for assay.

A 10-ml. portion of each of the above 50-ml. samples was titrated for

TABLE 1

Vitamin C in the stored milk

Date	Sample	Reduced ascorbic acid in original fresh milk	Reduced ascorbic acid in frozen milk	Cucumber enzyme frozen milk	*E. coli* frozen milk	Cucumber enzyme and *E. coli* frozen milk	Reduced ascorbic acid recovered*, per cent
		Vitamin C—milligrams per liter					
Feb. 19, 1940	1	40.80	34.74	0.67	37.41	22.38	59.82
	2	40.80	37.41	0.67	34.40	25.72	74.77
	3	40.80	34.74	2.00	35.07	23.71	67.61
	4	40.80	32.73	0.67	32.40	29.06	89.69
	5	40.80	43.41	10.43	39.03	32.64	83.63
	6	40.80	37.69	17.50	40.04	33.31	83.19
	7	40.80	39.71	22.88	42.06	33.99	80.81
	8	40.80	37.69	14.13	42.06	34.32	81.60
	9	40.80	39.17	1.34	39.47	30.44	77.12
	10	40.80	38.80	0.67	38.80	30.44	78.45
	11	40.80	43.15	0.67	38.80	32.11	82.75
	12	40.80	38.13	0.67	33.45	30.77	91.98
Feb. 20 1940	13	31.89	25.72	1.34	26.39	23.71	89.84
	14	31.89	29.06	1.34	30.06	28.06	93.34
	15	31.89	25.05	0.67	25.72	23.38	90.90
	16	31.89	27.72	0.67	28.72	26.72	93.04
	17	31.89	34.74	6.68	30.73	24.05	78.26
	18	31.89	30.73	5.34	29.06	24.72	85.06
	19	31.89	29.39	2.67	31.40	25.38	80.86
	20	31.89	26.72	2.67	26.72	22.04	82.49
	21	31.89	30.73	0.67	30.39	16.03	52.74
	22	31.89	30.39	0.67	30.39	17.37	57.16
	23	31.89	29.73	0.67	28.72	16.03	55.81
	Ave.	36.53	33.75		33.53	. .	. .
		Fresh milk plus additional vitamin C					
	1	66.80	..	0.00	67.47	60.12	89.11
	2	66.47	.	0.00	67.13	59.45	88.56
	3	66.47	..	0.00	67.80	59.45	87.68
	4	66.47	......	0.00	67.47	59.79	88.62

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* The reduced ascorbic acid recovered as recorded in column 6 is the percentage of reduced ascorbic acid in column 5 of that in column 4.

reduced ascorbic acid by the Sharp method (6). Another 10-ml. portion was analyzed for total vitamin C by the method of Gunsalus and Hand (3). To the remaining 30 ml. of the sample 0.2 ml. of cucumber concentrate (7) was added. This mixture of milk and cucumber juice was then aerated by shaking occasionally during a period of 15 minutes to convert the reduced ascorbic acid to the reversibly oxidized form. At this point another 10-ml. portion was titrated to ascertain the extent of the activity of the cucumber oxidase. Lastly, 10 ml. of the milk that was treated with the cucumber oxidase was treated with *E. coli* and analyzed for the total vitamin C content by the method of Gunsalus and Hand (3).

A substance that is oxidized by the cucumber oxidase and reduced again by *E. coli* to a compound which reacts instantly with sodium 2–6 dichlorobenzenoneindophenol presumably is ascorbic acid. No naturally occurring substance except ascorbic acid has yet been found that will respond to this test, although some are known which are oxidized by cucumber oxidase. Good recovery of ascorbic acid in milk is 85 to 90 per cent. Table 1 shows the results of the tests.

It should be noted that samples 1–12 were taken from the same milking on February 19, and that samples 13–23 likewise were taken from the same milking on February 20. According to the averages in this table the drop in the amount of reduced ascorbic acid in 2 years was 2.78 milligrams per liter of milk. It would be expected that the average of column 4 would be distinctly higher than column 2. Actually the reverse is true, with only a small difference. Perhaps this limited number of data should not be treated statistically.

The data reported in columns 3 and 6 of table 1 are the most significant. The cucumber oxidase should oxidize all the ascorbic acid. The data in column 3 indicate that samples 3, 5, 6, 7, 8, 17, 18, 19, and 20 have interfering substances, or reductones. The other samples are near blank readings and show, with little doubt, that the substance under inspection was vitamin C. It should be observed that the check samples of fresh milk in column 3 show complete oxidation.

As stated previously, the recovery of vitamin C, was found in column 6, should be as much as 85 to 90 per cent. The fresh milk checks confirm that statement. The recoveries of vitamin C in the 23 stored samples of milk show that 7 conform to that standard and that several others are near that point.

SUMMARY

The milk of each of the 4 cows in the first experiment was free from the oxidized and other off flavors at the beginning of the study. As the feeding of cod-liver oil progressed, the fresh milks lost their characteristic flavors of new milk. They became oily, but not the distinct cod-liver oil flavor. On standing, a "goaty" flavor appeared in the milks of cows 1, 2, and 3, whereas the oxidized flavor developed in the milks of cows 1 and 4.

In the second experiment the milks of cows 3 and 4, with only slight exceptions, did not become oxidized. The milks of the other 4 cows, on the other hand, became oxidized apparently as the result of administering cod-liver oil either in the feed or by drenching. During a period of 41 days of drenching, the first milk from cow 1 showed a slightly oily flavor. It was not typically fishy. Later, however, a very strong oxidixed flavor developed which was of the fishy order. The goaty flavor appeared in this experiment only once. It lasted from November 25 to December 4 in the milk of cow 4. In this experiment, the goaty flavor appeared during the latter part of a feeding period and the first few days of the following rest period. This also was true in the three examples of the previous year. The vitamin C, however, was below average when the milk was "goaty" in this experiment, whereas during the first year this flavor was noticeable when the vitamin C was present in abundance.

The following figures in the first experiment show the increases in reduced ascorbic acid in the milk from the check level when no cod-liver oil was fed, to the average of the three high readings when the amount of the oil was given at the highest level: Cow 1, 111 per cent; cow 2, 31.3 per cent; cow 3, 48.1 per cent; and cow 4, 38.3 per cent. The oil was given by drenching in this experiment.

There was no increase in reduced ascorbic acid in the second experiment when the cod-liver oil was mixed with the feed. In case of cow 2, possibly there was a slight increase during the period of 16 days when she received her cod-liver oil by drenching. Cow 1, on the other hand, responded in the same way that she did the first year, for the reduced ascorbic acid in her milk increased from an average of 25.48 mg. per liter during the 25 days preceding the drenching period to 66.43 mg. per liter on the peak day, or an increase of 163 per cent.

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THE RESULTS OF DEAERATION ON THE OXYGEN, VITAMIN C, AND THE OXIDIZED FLAVORS OF MILK

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The oxidized flavors of milk and milk products constitute one of the most studied problems in the field of dairy manufactures. This can be verified by observation of the review of literature dealing with the subject of oxidized flavors by Brown and Thurston (1), in which they listed 412 references. Much consumer complaint of pasteurized milk is not made because of the cooked flavor or the so-called "pasteurized flavor," but because of the oxidized flavors, which in the first stages of development may easily be confused with the cooked flavor. These flavors may be described as "papery," "oily," "metallic," "fishy," and "tallowy.".

It is known that the oxidized flavors are caused by chemical changes within the milk. Exposure of milk to daylight, under certain conditions, is one factor starting oxidation in the milk. A few metals, particularly copper, constitute a second factor since they act as catalysts in the development of the oxidized flavors. These flavors appear in the milk of at least one-third of the cows in the Cornell University herd before it is 7 days old, even though it is stored in brown-glass bottles for protection from daylight. This milk is pasteurized at 143° F. for 30 minutes, and it is stored at near 35° F. One set of 55 samples of milk from individual cows of the Cornell University herd in June, 1944, after seven days storage, was found to have the following oxidized-flavor scores[1]: 20 samples scored 0, 5 samples scored ?, and 30 samples scored 1 or above. On the 73rd day the results were: 5 samples scored 0, 3 samples scored ?, and 47 samples scored 1 or above. These data indicate that all milks will develop the oxidized flavors when held sufficiently long and in such a manner that there is little or no bacterial spoilage. It is claimed that an enzyme that survives the usual low-temperature pasteurization is a third factor in oxidized flavor development. The presence of air in the milk is a fourth factor. This fourth factor is the one that was under consideration in this study.

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[1] Oxidized flavors scoring system:

— = no oxidized flavor detected.
? = oxidized flavor doubtful.
1 = slightly oxidized.
2 = distinctly oxidized.
3 = strongly oxidized.
4 = very strongly oxidized.

When summarizing: — = 0, ? = 0.5, 1 = 1, 2 = 2, etc. Consumer complaints are likely to be made if the score is above 2.

The history of the oxidized flavors in the milk of Cornell University herds during a period of 23 months just prior to the deaeration studies reported in this paper is shown in table 1.

The data that are not recorded in table 1 but from which these monthly figures are taken show a great deal of variation from day to day. During these 23 months samples were taken on 470 days. The range in scores of the milk after a holding period at 35° F. for 3 days was 0 to 4. There were 77 samples that scored above 2.00. The range in the 7-day samples also was 0 to 4, with 262 of them scoring above 2.00 (262 consumer complaints).

TABLE 1

Oxidized flavors in the Cornell University milk previous to the installation of deaeration equipment

Date		Number daily samples in month	3 days old		7 days old	
Year	Month		Score*	Above 2.00*	Score	Above 2.00
1939	Nov.	23	0.60	0	2.45	11
1939	Dec.	20	0.88	1	2.72	9
1940	Jan.	27	2.04	10	3.63	25
1940	Feb.	14	1.96	4	3.61	13
1940	Mar.	24	2.06	10	3.42	17
1940	Apr.	23	2.78	16	3.86	22
1940	May	21	1.59	6	2.93	16
1940	June	.	.	.		.
1940	July	25	0.56	0	1.16	0
1940	Aug.	26	1.08	1	3.25	14
1940	Sept.	8	1.11	0	1.87	0
1940	Oct.	17	0.66	0	1.37	0
1940	Nov.	21	0.23	0	0.83	0
1940	Dec.	21	0.55	0	2.55	13
1941	Jan.	26	1.00	0	3.00	19
1941	Feb.	22	0.61	0	2.02	7
1941	Mar.	25	0.85	0	2.60	20
1941	Apr.	18	2.66	10	3.00	9
1941	May	28	1.31	5	3.27	24
1941	June	15	0.08	0	1.32	0
1941	July		.			.
1941	Aug.	19	0.19	0	0.97	3
1941	Sept.	7	1.00	0	2.57	4
1941	Oct.	21	1.89	7	3.14	18
1941	Nov.	19	2.06	7	3.21	18
	Total	470	. . .	77		262

* See footnote explaining flavor scoring.[7]

Apparently the general observation by dairymen that the oxidized flavors are less pronounced in the summer than during the other seasons is true.

One logical way to prevent the development of the oxidized flavors in dairy products is to eliminate the oxygen. When this is done, the other three factors, daylight, certain metals, and the oxidative enzyme, are inactive.

Two deaerating units were employed in this research. A smaller unit and the first one used is intermittent in operation. Inasmuch as this report deals for the most part with data obtained while using a larger unit, the only reference to this smaller equipment will be found in table 10. The larger unit, which is continuous, has a capacity of 3,000 pounds of milk an hour. The continuous deaeration is accomplished by flashing the milk into a stainless-steel vacuum chamber 18 inches in diameter and 52 inches deep.

The flow of the milk through this continuous deaerating equipment is as follows: From a 300-gallon vat the milk goes through a section of the short-time holding pasteurizer where the temperature is raised to approximately 115° F., which is well above the melting point of the fat. Fluidity of the fat when the milk is highly agitated, as when it is pumped or sprayed, is essential to subsequent satisfactory creaming. Next the milk, in the form of a spray, is drawn into the deaerating chamber in which about 29 inches of vacuum is maintained and where the temperature drops about 10° F. At this time approximately 1 per cent of the milk passes out in the form of vapor which sweeps out the oxygen as it goes. Only approximately 8 gallons of milk are in the deaerator at any one time. The milk, then, is drawn by a positive-action pump from the deaerator and is forced back through the pasteurizer where it is heated to 163° F. for 16 seconds and then cooled. From there it goes to the surge or supply tank that feeds the bottler. Very little air is reincorporated before the milk reaches the bottler.

At present, the milk is not bottled under vacuum. Plans for such a filler, however, have been made. When peacetime measures permit, there will be opportunity for the manufacture of this apparatus and of other refinements in the equipment.

In order to have some knowledge of the oxygen content of milk, determinations were made at various points in the production process. Limited data indicate that there is no oxygen in the milk in the cow's udder.[2] At the time the milk is removed from the udder, it can be assumed that due to atmospheric oxygen, chemical changes immediately start. A study of table 2 will reveal the comparative effect of hand milking and machine milking on the oxygen in the milk.

The data in the upper and lower parts of table 2 were obtained with different makes of milking machines. They were used in 2 different herds.

The amount of oxygen incorporated in the milk of several cows when milked by hand by different milkers can be observed in table 3.

These figures were obtained in one day. They are illustrative of the trials of 6 days. A comparison might be made with the "hand milked" column in table 2.

[2] This statement is made on the basis of only one short test. With the help of Dr. M. G. Fincher, of the Veterinary College, Cornell University; Paul F. Sharp and E. S. Guthrie obtained milk from all four quarters of one cow.

TABLE 2

Comparison of the effect of hand milking and the milking machine on the oxygen content of the milk

Cow	Hand milked	Machine milked	Difference	Hand milked	Machine milked	Difference
	Aug. 6	Aug. 7		Aug. 8	Aug. 9	
1	6.43*	4.48	1.95	6.63	4.70	1.93
2	5.63	5.25	0.38	7.44	5.46	1.98
3	6.76	4.91	1.85	6.97	4.70	1.77
4	5.94	5.04	0.90	5.80	4.83	0.97
5	5.63	4.42	1.21	4.54	4.64	–.10
6	5.76	4.26	1.50	3.57	3.91	–.34
Average			1.29			1.03
	Nov. 14	Nov. 15		Nov. 28	Nov. 29	
1	5.35	4.55	0.80	5.73	4.74	0.99
2	5.78	5.66	0.12	5.18	5.08	0.10
3	5.48	4.49	0.99	5.90	5.27	0.63
4	5.98	4.51	1.47	4.74	3.91	0.83
5	5.30	4.03	1.27	5.02	3.79	1.23
6	5.41	4.09	1.32	5.55	4.27	1.28
Average			0.99			0.84

* The figures are in terms of milligrams of oxygen per liter.

While endeavoring to ascertain how air is incorporated in the milk during the milking process, the results in table 4 were obtained.

The first method was the usual one of driving the stream of milk from the teat directly into the milk in the pail. The second method was to direct the stream against the sides of the pail. Less foam appeared in the latter method, and less oxygen was incorporated.

It seemed advisable to determine the maximum amount of oxygen that could be incorporated in milk under conditions of vigorous agitation in air. Studies on this point were made, one of which is reported in table 5.

A pint of milk from the filler was poured into a quart bottle and shaken in an up-and-down motion 50 times. Then it was poured back into a pint bottle, and after standing about 15 minutes a sample for oxygen analysis was drawn from the bottom. The temperature of the milk was approxi-

TABLE 3

Variations of oxygen in the milk of different cows

Cow	Milker	Comments	Oxygen
			mg./l.
1	1	1 inch foam, very easy milker	6.39
2	2	Easy milker, small streams	7.77
3	3	½ inch foam, easy milker	5.72
4	4	No foam, spray stream	6.75
5	5	No foam, easy milker	6.71
6	5	No foam, easy milker	6.57

TABLE 4

A comparison of methods of hand milking on the oxygen content of milk

Cows	1	2	3
First method	6.55*	6.03	5.05
Second method	4.91	5.64	4.89
Difference	1.64	0.39	0.16

* Milligrams per liter of oxygen.

mately 40° F. These analyses were made on consecutive days in January and February. Each analysis in this table represents an individual sample of milk.

It is important to know how much oxygen is incorporated in milk in the different stages of processing. Table 6 supplies this information.

TABLE 5

Maximum oxygen content of milk

Sample No.	Oxygen mg./l.	Sample No.	Oxygen mg./l.	Sample No.	Oxygen mg./l.	Sample No.	Oxygen mg./l.
1	9.09	7	9.98	13	9.46	19	9.41
2	10.35	8	9.74	14	9.42	20	9.71
3	10.51	9	9.20	15	10.47	21	9.27
4	10.11	10	9.54	16	10.56	22	9.16
5	10.52	11	9.75	17	9.43	23	9.79
6	10.54	12	9.43	18	9.72		

The amount of oxygen which may be found in milk before it reaches the deaerator is indicated in tables 5 and 6. With deaeration, when the outfit is working satisfactorily, the oxygen can be and has been reduced to zero point. During the period from January 1, 1942, to June 17, 1944, the analyses of 49 of 362 samples showed that the oxygen was completely eliminated and that many others were near that point. Such data set a bright future for the possible performance of new and improved equipment. This

TABLE 6

*Oxygen content of milk in the different steps in handling**

Stages	Oxygen	Stages	Run 3 oxygen	Run 4 oxygen
	mg./l.		*mg./l.*	*mg./l.*
Patrons' cans	6.09	Raw milk to balance tank	11.9	10.0
Weigh tanks	7.05	Entering exchanger	11.0	9.9
Pump reservoir	7.65	161° F. beginning hold	7.1†	5.9†
Tank cars	9.19	End of hold	6.1†	6.0†
Tank truck	9.68	Leaving cooler	10.2	9.8
		Bottle	11.2	10.4

* Composite of tables 1 and 5 by Sharp, Guthrie and Hand (2).

† Owing to high temperatures, air was lost from the milk as soon as it was liberated to the outside atmosphere.

is especially impressive when it is realized that the daily peak load of this small deaerator was 20,000 bottles of milk, that it now has been in operation for 3½ years, and that a large part of the time it has been operated by two high school boys and other untrained personnel. It should be explained that most of the bottles were the one-half pint size.

Concerning the operation of the deaerator, inquiry often is made about the uniformity of deaeration. The answer to that question is found in table 7.

This operation, of course, was in the continuous flow unit. It was working smoothly and apparently in a normal way. The oxygen content, however, was not sufficiently low. That was the reason for this experiment.

The effect of deaeration on the vitamin C content of the milk was closely observed in this study. One way of determining the results of the removal of the oxygen was to see what happened to the vitamin C content of stored milk during the season when the oxidized flavors were intense. Table 8 shows what took place in one such period in milk that was processed in the continuous flow outfit.

TABLE 7

Variations in the oxygen content of deaerated milk taken at intervals of 15 minutes

Sample	Oxygen	Sample	Oxygen	Sample	Oxygen
	mg./l.		*mg./l.*		*mg./l.*
A	0.87	C	0.87	E	1.50
B	0.95	D	0.65	F	1.06

The oxidized flavors of the same samples of milk that are listed in table 8 are tabulated in table 9.

Table 9 shows that deaeration has much to do with the prevention of the development of the oxidized flavors in milk. These data, it should be recalled, were obtained from milk processed in the commercial-continuous flow outfit.

One of the long-time experiments of this research was that of maintaining the vitamin C and the flavor for one week in the milk that was used in a child-feeding study in the New York State College of Home Economics at Cornell University. The milk was supplied by the Department of Dairy Industry for a period of approximately 20 weeks in each of three consecutive years.

The results of the experiments are shown in table 10. The data of the first year and the grand averages of all three years are presented. The milk was taken from the general supply of the Department, which at that time was practically all produced on the University farms and was a mixture of the milk of the three milkings of each day. It was pasteurized when obtained. After deaeration in the small intermittent-flow unit, the milk was bottled by the in-bottom method[3] and was held at the usual milk-storage temperatures of 35° to 40° F.

It should be observed that in table 10, the reductions in vitamin C in seven days in the deaerated and undeaerated milks were 0.85 and 10.78 milligrams per liter, respectively, and that the deaerated milk was "good" in flavor, whereas the undeaerated milk was "poor." The grand averages for the three years show that the "fresh deaerated," "seven-day deaerated,"

TABLE 8

Deaeration and vitamin C in milk

Date April 1943	Vitamin C* fresh undeaerated	Age in days	Vitamin C		
			Deaerated		Air reincorporated§
			In-bottom† filled	Commercially‡ filled	
	mg./l.		*mg./l.*	*mg./l.*	
5	16.31	3	16.24	14.97	13.38
		7	15.51	12.66	6.01
8	18.47	3	16.48	16.48	14.90
		7	14.95	14.63	5.41
12	17.72	3	15.24	14.92	10.80
		7	16.41	13.88	4.73
16	17.14	3	17.04	17.35	17.04
		7	17.96	17.23	11.80
19	18.30	3	16.93	17.57	17.57
		7	17.22	16.27	13.40
22	19.81	3	17.57	18.85	16.61
		7	18.87	17.61	9.75
26	18.04	3	16.67	16.67	13.84
		7	17.39	14.28	5.28
29	20.13	3	18.94	18.01	14.90
		7	16.91	15.99	5.54
Average	18.24	3	16.89	16.85	14.88
		7	16.89	15.32	7.74

* "Fresh undeaerated" is the original milk. It should be understood that about one-half of it, a part of the time, was purchased from outside the Department and held in the raw condition one day, so some of the milk was almost 2 days old when it was processed with the University supply which was 18 hours old or younger, depending on when it was milked.

† "In-bottom filled" has reference to the way that the deaerated milk was put into the bottles. As the milk flowed through a glass tube to the bottom of the bottle, the air was forced up and out of the bottle. Thus only a little oxygen was reincorporated in the milk.

‡ "Commercially filled" has data on the deaerated milk that was bottled under atmospheric conditions where about 2 milligrams of oxygen per liter were reincorporated in the milk.

§ "Air reincorporated" were samples of "commercially filled" cold milk that were thoroughly shaken in order to fill them with oxygen. This milk then was considered to be the check or control sample. It cannot correctly be termed "undeaerated." The samples were obtained in this manner because in the commercially continuous operation it was not possible to take undeaerated and deaerated samples of exactly the same milk. They were more nearly the same when taken in this way.

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[3] See footnote (†) table 8.

and "seven-day undeaerated" contained 17.79, 16.42, and 7.68 milligrams per liter of vitamin C, respectively, and that the flavor scores were practically the same as those obtained in the first year.

The figures in table 11 are the averages of 250 samples, only one a day, in two and one-half years.

TABLE 9

Deaeration and the oxidized flavors in milk

Date April 1943	Oxygen* fresh deaerated	Age in days	Flavor			
			Deaerated		Air reincorporated**	Fresh undeaerated†
			In-bottom filled**	Commercially filled**		
	mg./l.					
5	0.12	3	– – – ‖	– – –	– – –	– – –
		7	? ? ? §	? ? ?	2 2 2	– – –
8	0.00	3	– – –	– – –	– – –	– – –
		7	– – –	? ? ?	2 2 2	– – –
12	0.09	3	– – –	? ? ?	1 1 1	– – –
		7	– – –	2 2 2	3 3 3	– – –
16	0.34	3	– – –	– – –	– – –	– – –
		7	? ? ?	– ? ?	? ? ?	– – –
19	0.16	3	– – –	– – –	– – –	– – –
		7	– – –	? 1 –	? 1 1	– – –
22	0.16	3	– – –	– – –	– – –	– – –
		7	? ? ?	– – –	2 2 2	– – –
26	0.46	3	– ? –	? – ?	1 1 1	– – –
		7	? ? ?	2 2 2	3 3 3	– ? –
29	0.10	3	– – –	– – –	– – –	– – –
		7	? – ?	? – –	3 3 3	– – –
Average	0.18	7	0.29	0.75	2.04	–
Average		7	39 (good)	36 (fair)	31 (poor)	40‡ (excellent)

* "Fresh deaerated" is the original milk immediately after deaeration.

** See footnotes, table 8.

† Same as (*) in table 8.

‡ American Dairy Science Association system of scoring. For convenience, the author has made the following groupings of flavor scores. Excellent 40 or above, without criticism; good 38–39; fair 35–37; poor 30–34; and bad 25–29.

§ The so-called blind system of judging was employed. Each sample was divided in 3 portions, and each portion was poured into a flavoring glass that was numbered on the bottom. Then all 12 flavoring glasses were shuffled. Thus the judge was "blind" so far as the identity of the samples was concerned. Most of the time the decisions were made easily. Occasionally, however, as on April 19, the 7-day sample was confusing, for the score was "? 1 –."

‖ Oxidized flavors system of scoring. See footnote 1.

Under "fresh" in table 11 there were 13 samples that showed an indication of the oxidized flavors. This is not surprising for, as stated in footnote *, table 8, some of the "fresh" milk was about two days old. In the "in-bottom filled" column, 107 samples had oxidized flavors on the seventh day, with 2 so poor that they would not have been accepted by the consumers. Among the "commercially filled" samples, 135 showed the

TABLE 10

Deaeration of the milk by the intermittent method

Fresh deaerated			Seven-day deaerated			Seven-day undeaerated		
Date	Vitamin C*	Score†	Vitamin C	Score	Comments	Vitamin C	Score	Comments
Jan.								
6	17.79	41	15.18	38.5	Slightly old	6.46	32	Oxidized
13	19.05	41	18.87	38.5	" "	9.77	29	"
20	16.85	40		...	...	..		...
22	16.30	41	15.79	38.5	Slightly old	9.20	32	Oxidized
28	18.45	40	14.85	40.0	...	6.70	32	"
Feb.								
3	17.67	40	17.89	39.0	Slightly old	6.96	32	Oxidized
10	17.99	40	16.27	40.0	" "	9.37	33	"
16	18.24	40	17.05	39.0	" "	7.05	32	"
23	16.46	40	15.74	39.0	" "	10.11	33	"
Mar.								
1	16.14	41	15.45	37.0	Slightly oxi.	5.64	32	"
8	16.48	41	18.38	40.0		6.13	32	"
15	19.91	41	20.87	40.0		6.11	32	"
22	18.83	41	17.32	40.0	...	7.15	32	"
29	17.83	41	14.68	40.0		2.39	32	"
Apr.								
5		41	17.78	38.5	Slightly old	6.16	29	"
12	18.81	41	17.91	38.5	" "	7.58	29	"
19	17.91	41	17.25	38.5	" "	5.86	29	"
28	17.94	41	16.80	40.0		6.66	32	"
Ave.	17.79	40.7 (excellent)	16.94	39.12	Good	7.01	31.4	Poor

* Milligrams per liter of vitamin C in terms of reduced ascorbic acid.

† Amer. Dairy Sci. Assn. system of scoring, table 9, footnote (‡).

oxidized flavors on the seventh day. The runs of only 15 days would have caused consumer complaint. It is understood, of course, that not much of this milk is held for seven days by the customers. The last column, which shows the effect of reincorporation of air, had 175 oxidized samples with 85 in the consumer complaint category.

The key to the flavor evaluations, in table 11, is in footnote [1]. In terms of proportion the three-days' readings would be: "In-bottom filled": "commercially filled": "air reincorporated": : 1: 2.5: 7. It could be stated,

TABLE 11

A comparison of the vitamin C and oxidized flavors in fresh and processed milks on 250 days during a period of two and one-half years

Fresh		Age	In-bottom filled		Commercially filled		Air reincorporated	
Vit. C	Flavor		Vit. C	Flavor	Vit. C	Flavor	Vit. C	Flavor
mg./l.			*mg./l.*		*mg./l.*		*mg./l.*	
17.19	0.0066	3 days	15.89	0.0372	15.22	0.0892	12.14	0.253
....	....	7 days	15.26	0.2526	13.03	0.611	6.08	1.421

therefore, that the intensity of the oxidized flavors in the “commercially filled” samples was 2.5 times that of the “in-bottom filled” samples, and that the intensity of the oxidized flavors in the “air-reincorporated” milks was 7 times greater than that of the “in-bottom filled” milks. Likewise, in the 7-day observation the proportion is: “In-bottom filled”: “commercially filled”: “air reincorporated”: : 1 : 2.5 : 5.5.

The evaluation of deaeration relative to the flavors may be observed in table 12. These judges are recognized critics of the flavors of milk. Their scores, therefore, make a satisfactory summary to the data on flavors.

TABLE 12

*The effect of deaeration of milk on the oxidized flavors**

Exhibited at Vermont Dairy Plant Operators' and Managers' Convention, October 25–26, 1939			
Set I—12 days old	*O. E. Herreid*	*J. A. Newlander*	*E. S. Guthrie*
Check sample	31 oxidized	31 oxidized	28 oxidized
Deaerated sample	39 slightly “off”	39 slightly “off”	38 slightly coppery
Set II—9 days old			
Check sample	33 oxidized	33 oxidized	31 oxidized
Deaerated sample	40 no criticism	40 no criticism	40 no criticism
Exhibited at the Metropolitan Dairy Technological Society, April 16, 1940			
Set I—8 days old	*H. F. Judkins*	*James Bohn*	*E. S. Guthrie*
Check sample	28 or 29 oxidized	28 or 29 oxidized	28 oxidized
Deaerated sample	40 no criticism	40 no criticism	40 no criticism
Exhibited at Massachusetts State College, May 9, 1940			
Set I—6 days old	*H. G. Lindquist*	*M. J. Mack*	*E. S. Guthrie*
Check sample	31 oxidized	31 oxidized	29 oxidized
Deaerated sample	40 no criticism	40 no criticism	40 no criticism
Set II—7 days old			
Check sample	31 oxidized	31 oxidized	29 oxidized
Deaerated sample	40 no criticism	40 no criticism	40 no criticism
Set III—29 days old			
Check sample	25 oxidized	28 oxidized	26 oxidized
Deaerated sample	39 slightly old	39 slightly unclean	39 lacked freshness
Exhibited at the University of Tennessee, January 27–29, 1942			
Set I—40 days old	*J. H. Erb,* Ohio State University		*E. S. Guthrie*
Check sample	33 oxidized		31 oxidized
Deaerated sample	39 slightly old		39 lacked freshness

* These data, with the exception of the exhibit at the University of Tennessee, were published in 1940 (3).

SUMMARY

1. The oxygen of milk may vary from 0, or near that point in the udder, to about 11 milligrams per liter in the bottle in undeaerated milk.

2. An intermittent deaerator was used in a series of experiments that extended through 3 periods in 3 years, or a total of 57 weeks. The vitamin C, in the milk in these series of analyses, dropped from 17.9 milligrams per liter in the fresh milk to 16.42 milligrams per liter in the deaerated milk at the end of 7 days, and to 7.68 milligrams per liter in the undeaerated milk during the same period.

3. The flavor of the milk that was deaerated in the intermittent unit was "excellent" when fresh. At the end of 7 days the deaerated milk was "good," whereas the undeaerated milk was "poor" due to the oxidized flavors.

4. A continuous deaerator has been in daily operation by largely unskilled operators for 45 months. Data obtained during the first thirty month's operation are reported. Forty-nine samples out of 362 analyses on as many days, contained no oxygen, and many others were near that point.

5. During the 30 months of the operation of the continuous deaerator the average of 250 analyses of fresh milk was 17.19 milligrams of vitamin C per liter. The "in-bottom filled," the "commercially filled," and the "air reincorporated" samples of 7 days' old milk averaged 15.26, 13.03, and 6.08 milligrams of vitamin C per liter, respectively.

6. The 250 comparisons made during the 30 months of daily operation of the continuous deaerator show that the flavor of the fresh milk, in general, was "excellent," and at the age of 7 days the "in-bottom filled," the "commercially filled," and the "air reincorporated" milks were good, fair, and poor, respectively.

7. The deaerators used in this study were constructed to take the oxygen out of milk in order to prevent the development of the oxidized flavors. The opinions of several reputable judges of milk show that they accomplished the purpose for which they were made. An added advantage of importance is the preservation of substantial amounts of vitamin C.

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RELATION OF THE TEMPERATURE OF SEPARATION AND THE HEAT TREATMENT GIVEN THE SKIM MILK TO THE KEEPING QUALITY OF SPRAY DRIED ICE CREAM MIX

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Previously reported studies (5, 20, 23) have shown that in so far as whipping ability, body and texture, and flavor are concerned, a very palatable ice cream can be made from fresh reconstituted powdered ice cream mix. A further desirable feature for dried ice cream mix is that there be no appreciable flavor deterioration for several months after manufacture. The most common defect that occurs during storage of the dry mix is the development of an oxidized flavor.

The industry has found that the use of a high preheating temperature before condensing is helpful in retarding the development of an oxidized flavor in powdered milk. Although the composition of milk "agglutinin" has never been reported, it is closely associated with the fat and can be concentrated either in the cream or skim milk depending on the temperature at which the milk is separated (22). In some plants it is a practice to separate milk cold (below 90° F.), while in other plants milk is separated hot (above 120° F.). Since it has been shown that the temperature at which milk is separated is related to the distribution of some of the minor constituents in milk such as "agglutinin" (22), it was thought advisable to study the relation of the temperature of the milk when separated and the forewarming temperature of the skim milk before condensing to the keeping qualities of the dried ice cream mix.

REVIEW OF LITERATURE

Holm, Greenbank, and Deysher (12) found that improved keeping quality of the dried whole milk resulted from forewarming the milk at higher than ordinary preheating temperatures (83° C. for 30 minutes). Jack and Henderson (13) also found that high preheating temperatures were beneficial. Hollender and Tracy (11) found that roller milk powder made from milk heated to 170° F. for 30 minutes was less likely to develop an oxidized flavor during storage than that made from milk heated to a higher (190° F.) or lower (150° F.) temperature for the same length of time. Mattick and coworkers (16) conclude that "increasing the preheating temperature of the milk from 165° F. to 190° F. before spray drying greatly improved the resistance of the powder to the development of tallowy 'off' flavors on storage." They suggest that the protective effect of the high preheating

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temperature is due (a) to production in the milk of antioxidant-active sulphydryl compounds, by the action of heat on the proteins and possibly (b), to the more complete destruction of oxidizing enzymes originally present in the milk or produced therein by the growth of bacteria.

Kende (15) found that the oxidative taint which develops in some milks, particularly at low storage temperatures, could be prevented by heating the milk at 185° F. for 5 minutes. He further states that reducing substances were formed in milk heated to 176–185° F.

Trout (24) recommends that cream to be stored frozen must be pasteurized at least at an exposure of 165° F. for 15 minutes and preferably 185° F. for five minutes. Dahle, Lawhorn .and Barnhart (4) concluded that a pasteurization temperature of 150° F. for 30 minutes offered no protection during storage if the copper content exceeded 0.6 p.p.m. A flash temperature of 170° F. offered protection in creams having a copper content over 0.75 p.p.m., but under 1.12 p.p.m., while 190° F. flash was only slightly better than 170° F.

Schieb, Stark, and Guthrie (21) have found that high temperature treatment of churning cream aids in producing butter resistant to the development of oxidative taints during storage at 0° F. to −10° F. They found that 165° F. for thirty minutes was necessary, 15 minutes at 165° F. was inefficient, and that cream itself is much more resistant to spoilage when flash-pasteurized at 170–190° F. than when heated to 150° F. for as long as 30 minutes.

There is conflicting evidence in the literature regarding whether the butterfat, fat globule membrane, copper or ascorbic acid are involved in oxidized flavor development. Brown and Thurston (2) have reviewed the various theories of the mechanism of fat oxidation. Since this review Olson and Brown (17, 18) have added some more possible explanations on oxidized flavor development and inhibition. El-Rafey, Richardson, and Henderson (6) have shown that improved keeping quality of butter oil results when the butter is heated to 110° C. to drive off the moisture than when lower temperatures of isolation of the oil are used. They attribute the improved keeping quality to result from the transfer of greater amounts of phospholipid material from the non-oil phase of butter to the oil, presumably because of the denaturing action of heat on the protein of the phospholipid-protein complex. The concentration of reducing substances is also higher in the butter oil made by the "boiling off" process than by the low heat treatment.

Dahle and Josephson (3) found that samples of dry whole milk containing the least lecithin had the best keeping qualities.

EXPERIMENTAL METHODS

The experimental batches were prepared and dried in a commercial plant equipped with a pilot drying unit of 75 pounds per hour capacity.

This plant had practically all stainless steel equipment. All batches of dried ice cream mix within a single run were made from the same lot of milk.

Fresh raw milk was separated at both 55° F. and 130° F. and the cream and skim milk collected to obtain cream and skim milk containing different concentrations of "agglutinin" (22). Theoretically the cream separated at 55° F. should contain most of the "agglutinin" originally present in the milk whereas the 55° F. skim milk should contain a relatively low concentration of "agglutinin." The milk separated at 130° F. should produce skim milk containing most of the "agglutinin" while the cream separated at 130° F. should be relatively low in "agglutinin" (22). Two 5000-pound batches of skim milk separated at the two temperatures (55° and 130° F.) were used. Portions of each batch were forewarmed at 150° F. for 20 minutes and 180° F. for 5 minutes and immediately condensed in a stainless-steel vacuum pan, cooled to 40° F. and stored overnight before use. All the cream was pasteurized at 170° F. for 10 minutes, cooled to 40° F. and stored overnight.

Butter was prepared from 40.5 per cent cream separated at 87° F., pasteurized at 170° F. for 10 minutes, cooled to 40° F. and held four hours before churning. Butter oil was made from the above butter by the decantation method. The butter was melted at 140° F. and held at this temperature overnight. The butter serum was then siphoned from the bottom and the scum removed from the surface. The fat layer was then filtered through cheesecloth.

Ice cream mixes having the composition of 10 per cent butterfat, 10.8 per cent m.s.n.f., 14.5 per cent sugar and 0.3 per cent stabilizer were made in 100-pound batches. The proper amounts of cream, condensed skim, water, sugar and stabilizer were mixed in a small preheater heated to 150° F., homogenized at 1500 pounds pressure and spray-dried at 2000 pounds pressure. Only one-fourth of the sugar was added before drying. When butter or butter oil was used as a source of fat, the desired amount of fat was first mixed with 5 pounds of condensed skim milk and 10 pounds of water, heated to 140° F. and homogenized at 1500 pounds pressure. It was then added to the remainder of the mix and processed as above.

The ice cream mixes were dried on a stainless-steel spray-type drier using a number 64 nozzle with a number 17 core. The air inlet temperature was approximately 310° F. and the outlet temperature 195–200° F.

After drying the mix the remainder of the sugar was added. Powdered vanilla was added at the rate of 3 ounces to 16 pounds of ice cream powder. The powdered mix was then packed in number 2 cans. Three hundred grams (plus or minus 2 grams) of powdered mix was added to each can. The cans were then sealed, nitrogen-packed and stored in a room where the temperature was automatically controlled at 72° F. except during the months when the temperature outdoors was higher than that indoors.

Changes in carbon dioxide and oxygen concentration in the head space were obtained by the manometric procedure of Van Slyke and Sendroy (25).

The copper content of the powdered mixes was determined by the carbamate method of Hetrick and Tracy (10) and the iron content by the 1,10-phenanthroline method of Pyenson and Tracy (19).

The flavor scores were determined on reconstituted ice cream mix by two or more judges within 8 hours after reconstituting. The ice cream score card with a perfect flavor score of 45 was used for scoring the reconstituted ice cream mixes.

Moisture was determined by the toluol distillation method (1).

The fat source and treatment given the ingredients used in making up the ice cream mixes before drying are indicated in table 1. The fresh milk was held in large storage tanks before separation. The same source of milk was used for the fat and skim milk. Four batches were made with butter oil as the source of fat and combined with skim milk separated at the two temperatures (55° and 130° F.) (table 1, samples 23A–23D). Four batches were also made with cream pasteurized at 170° F. for 10 minutes at both 55° F. and 130° F. and combined with condensed skim milk forewarmed at 150° F. for 20 minutes or 180° F. for 5 minutes to vary "agglutinin" content and heat treatment of the milk solids.

TABLE 1

Fat source and treatment of ingredients in ice cream mixes

Batch No.	Fat source	Cream treatment			Skim milk treatment		
		Separation temp.	Past. temp.		Separation temp.	Forc. temp.	
		°F.	*°F.*	*min.*	*°F.*	*°F.*	*min.*
23A	Butter oil				55	150	20
23B	Butter oil				55	180	5
23C	Butter oil				130	150	20
23D	Butter oil				130	180	5
23E	Cream	55	170	10	130	150	20
23F	Cream	130	170	10	55	150	20
23G	Cream	130	170	10	130	150	20
23H	Cream	130	170	10	130	180	5
24A	Butter oil				87	150	20
24B	Butter				87	150	20
24C	Cream	87	170	10	87	150	20
24D	Cream	87	170	10	87	180	5

Batches number 24A–24D were run simultaneously with batches 23A–23H using the same products to permit comparison between different lots of both batches. In these samples butter oil, butter and cream as the source of fat were compared using skim milk separated at 87° F. and forewarmed at 150° F. for 20 minutes before condensing. One batch (24D) was made with condensed skim milk forewarmed at 180° F. for 5 minutes using cream separated at 87° F. as the source of fat.

STORAGE OBSERVATIONS ON DRIED ICE CREAM MIX

Table 2 indicates the changes in carbon dioxide and oxygen in the head space gas and palatability of gas-packed dried ice cream mix on storage for one year at room temperature.

TABLE 2

Changes in carbon dioxide and oxygen in head space gas and palatability of gas-packed dried ice cream mix on storage

Batch No.		Days of storage at room temperature						
		22	52	110	170	210	300	365
23A	% CO_2	0.75	0.31	0.32	0.67	1.02	1.06	1.22
	% oxygen	2.64	2.24	2.07	1.60	0.95	0.53	0.41
	Flavor	38*	36*	35*	35*	35*	35*	34*
23B	% CO_2	0.42	0.28	0.33	0.80	0.50	0.54	0.23
	% oxygen	2.93	3.00	2.56	2.33	2.25	1.44	1.43
	Flavor	40.5	40	39.5	39	38†	38.5	38
23C	% CO_2	0.71	0.38	0.91	0.87	0.61	0.52	0.81
	% oxygen	2.27	3.49	1.86	2.33	1.78	0.85	0.51
	Flavor	40.5	40	38*	37*	38	37*	36.5*
23D	% CO_2	0.91	0.38	0.57	0.65	0.81	0.61	0.63
	% oxygen	2.91	3.00	1.78	2.16	1.86	1.11	0.87
	Flavor	40.5	39.5	39.5	39	39	38.5	38.5
23E	% CO_2	0.53	0.66	0.48	0.81	1.06	1.01	1.23
	% oxygen	2.57	2.54	2.33	2.04	1.69	1.11	0.59
	Flavor	40.5	39.5	36†	36†	36.5*	35.5*	35*
23F	% CO_2	0.74	0.55	0.84	1.16	0.93	1.50	0.95
	% oxygen	3.49	3.04	2.19	1.80	1.63	0.93	0.74
	Flavor	40.5	38	34.5†	35†	35*	35*	35.5*
23G	% CO_2	0.75	1.07	0.72	0.73	1.13	1.21	1.05
	% oxygen	2.56	2.88	3.00	2.36	1.55	1.10	1.03
	Flavor	40.5	37.5*	37.5	34.5*	37*	35*	36.5*
23H	% CO_2	0.53	0.29	0.44	0.49	0.46	0.62	0.87
	% oxygen	3.87	2.91	2.47	2.43	2.31	1.22	1.97
	Flavor	40.5	40	39.5	39	39	39	38.5
24A	% CO_2	0.94	0.00	0.53	0.41	0.82	1.03	0.97
	% oxygen	2.22	2.39	1.82	0.65	0.76	0.33	0.65
	Flavor	40.5	38*	37*	34.5*	36*	35.5*	34.5*
24B	% CO_2	0.72	0.15	0.36	0.63	0.84	0.94	0.93
	% oxygen	2.55	2.40	2.03	1.53	1.45	0.82	0.71
	Flavor	40.5	40	37*	35*	36*	35*	35*
24C	% CO_2	1.08	0.81	0.92	1.13	1.30	1.59	1.33
	% oxygen	2.76	2.72	2.36	1.63	1.35	0.72	0.45
	Flavor	39	38.5*	36†	34.5*	35*	34.5*	34.5*
24D	% CO_2	0.69	0.76	0.56	0.48	1.11	1.10	0.95
	% oxygen	2.70	3.16	2.40	0.42	1.86	1.09	0.81
	Flavor	39	39.5	39	38.5	38	37.5	38

* Oxidized.
† Metallic.

The carbon dioxide content of the head space gas varied throughout the storage period. A relatively large amount of carbon dioxide developed and remained throughout the intervals of analysis. Oxygen concentration in the headspace gas gradually became lower as the storage period advanced.

At 22 days of storage, the oxygen values varied from 2.27 per cent to 3.87 per cent. At the end of one year the oxygen values decreased more than 50 per cent. In most cases more than two-thirds of the oxygen in the headspace was absorbed during the year of storage.

The flavor scores in table 2 indicate that, in general, the powder became less palatable as the storage period advanced. The initial oxygen content of the headspace gas was lowest in those cans containing powder made with butter oil as a source of fat. Nevertheless the keeping qualities of these mixes were no better than those made with cream. There was slightly more rapid oxygen absorption, more carbon dioxide evolution and more oxidized flavor development in mixes made from the condensed skim milk forewarmed at 150° F. than in those mixes that had the condensed skim milk forewarmed at 180° F. When the skim milk was forewarmed at 180° F. for 5 minutes. the reconstituted dried mix developed a flavor designated as stale or lacking in freshness, but the typical oxidized flavor was not apparent.

TABLE 3

The moisture, iron and copper content of spray dried ice cream mix

Batch No.	Moisture	Iron	Copper
	%	*p.p.m.*	*p.p.m.*
23A	0.8	4.4	0.65
23B	1.0	3.3	0.58
23C	0.8	3.6	0.93
23D	1.0	3.7	0.55
23E	1.1	3.6	0.53
23F	1.4	3.9	0.58
23G	1.2	3.8	0.65
23H	0.8	3.4	0.65
24A	0.8	3.6	0.60
24B	1.6	3.2	0.68
24C	0.8	4.0	0.70
24D	1.5	3.4	0.65

Higher flavor scores occurred in the reconstituted ice cream mixes when the skim milk was forewarmed at 180° F. for 5 minutes. In every case, regardless of the source of fat, forewarming the skim milk before condensing to a high temperature prevented the development of oxidized flavor during the storage period (12 months). The temperature at which the milk was separated did not affect the keeping quality or oxidized flavor development. From table 2 it can be seen that batches 23B, 23D, 23H and 24D which were made from skim milk forewarmed at 180° F. developed no oxidized flavor and were almost as palatable at the end of the storage period of 12 months as they were at the beginning of the experiment.

From the results summarized in table 2 and from the above discussion, it can be seen that high forewarming treatment on the skim milk used as a source of solids is beneficial in prolonging the keeping quality of dried ice cream mix. This phenomenon may be associated with the liberation of the sulfhydryl groups on high forewarming treatment. Some heat labile sulphides are liberated at the high forewarming temperature used in this study (6, 7, 8, 14). The temperature of separation of the cream and the source of fat, whether butter oil, butter or cream, made little difference on palatability. Further work is now in progress to secure information on the relation of the heat treatment given the butter fat to the keeping quality of spray dried ice cream mix.

THE MOISTURE, IRON AND COPPER CONTENT OF SPRAY DRIED ICE CREAM MIX

A summary of the moisture, iron and copper content of the mixes reported in this investigation are given in table 3. The moisture in all the dried mixes was 1.6 per cent or less. The iron varied in the various batches from 3.2 to 4.4 parts per million and the copper varied from 0.55 to 0.93 parts per million.

SUMMARY

Twelve batches of dried ice cream mix were made from the same lot of milk. The milk was separated at various temperatures to secure cream and skim milk containing various amounts of "agglutinin." The skim milk was forewarmed at both low and high temperatures and condensed in a stainless steel vacuum pan. Some of the cream was made into butter and butter oil. Dried ice cream mixes were made from these ingredients so that the relation of the heat treatment given the serum solids to the keeping quality could be studied.

The carbon dioxide content of the headspace gas varied throughout the storage period. Oxygen concentration gradually became lower as the storage period advanced. There was a tendency toward more rapid oxygen absorption, more carbon dioxide evolved, and more oxidized flavor development in mixes that had the condensed skim milk forewarmed to 150° F. for 20 minutes than in those mixes that had the condensed skim milk forewarmed at 180° F. for 5 minutes. There seemed to be no significant differences in oxidized flavor development when the mixes were prepared from concentrated skim milk made from skim milk separated at 55° or 130° F. Whether the fat used was in the form of cream, butter or butter oil was not a factor influencing the keeping quality of the dried ice cream mix. All the batches became oxidized which contained butter oil, butter or cream which were made with skim milk forewarmed at 150° F. for 20 minutes. After one year dried mixes made from skim milk forewarmed at 180° F. for 5 minutes had not developed an oxidized flavor and were still highly palatable.

High forewarming temperature on the skim milk before condensing is beneficial in prolonging the keeping qualities of dried ice cream mixes and the retardation of oxidized flavor development.

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CAROTENE UTILIZATION IN THE NEWBORN CALF

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Mature bovine obtain their vitamin A requirements from the metabolic conversion of carotene because usual rations do not contain preformed vitamin A. On the other hand, the newborn calf normally receives considerable amounts of vitamin A and carotene from colostrum. Sutton (12) reports that calves receive about 53 milligrams of carotene when fed colostrum during the first seven days. If it is utilized, this carotene is an important source of vitamin A. However, little is known regarding the ability of the newborn calf to utilize carotene. Suggestions that the newborn calf is unable to utilize carotene (9), or that the young calf converts carotene more efficiently or more rapidly than older animals (7) have been made, although few data have been presented. Therefore, it is important to know if the newborn calf utilizes carotene and the age at which conversion is sufficient to provide the full requirement of vitamin A.

The study, reported herein, was undertaken to obtain more specific information on this problem.

EXPERIMENTAL

Male calves of the Holstein, Guernsey, and Jersey breeds were used. Because of the high vitamin A and carotene content of colostrum, the calves were fed fresh skim milk or reconstituted skim milk from birth. A low-carotene ration composed of ground oats, ground barley, wheat bran, linseed oil meal, dicalcium phosphate, and salt was fed *ad libitum*. Dried beet pulp was included in the ration as the sole source of roughage. In order to minimize the possibility of vitamin deficiencies other than vitamin A, each calf received daily a capsule[1] containing 1000 U.S.P. units of vitamin D, 250 milligrams of ascorbic acid, 50 milligrams of alpha-tocopherol, 10 milligrams each of thiamine, riboflavin and pyridoxine, 100 milligrams of niacin, 50 milligrams of calcium pantothenate and 1000 milligrams of choline chloride.

Various materials were used to supply carotene. During the early phases of the study, solutions containing one gram of crystalline carotene[2] dispersed in 100 ml. of peanut oil or 500 ml. of lard were used. Alfalfa leaf meal containing 12.8 mg. of carotene per 100 grams was also used. A commercial concentrate[3] containing 33 mg. of carotene per gram derived from vegetable sources and dispersed in cottonseed oil was used during the latter part of the experiment. The calves were weighed at weekly intervals, and the carotene intakes were adjusted according to body weight.

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[1] Generously supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

[2] 90% Beta-carotene. General Biochemicals, Chagrin Falls, Ohio.

[3] Carotene in oil, Type 288. General Biochemicals, Chagrin Falls, Ohio.

The calves were housed in individual stalls in a separate barn that was provided with artificial heat in cold weather to avoid the complicating effects of extremes in temperatures.

Plasma carotene and vitamin A were determined at birth and at least twice weekly thereafter using the method of Kimble (8). Ophthalmoscopic examination of the eyes were made at frequent intervals. All calves were slaughtered at 60 days of age and a careful examination made of the various organs for gross pathological conditions. Analyses of the livers were made according to the extraction procedure of Davies (3) to determine the storage of carotene and vitamin A. Similar examinations and analyses were made of all calves that died during the course of the experiment.

During the early phases of this study a transfusion of about 500 ml. of the dam's blood was given to each calf at birth. In some cases an additional 250 ml. of plasma or blood was injected subcutaneously. This practice was recommended by Smith and Little (11) as an effective means of providing the protective immune bodies normally supplied by colostrum. This procedure proved to be impractical and unsatisfactory as a prophylactic measure and was replaced by the oral administration of 4 to 8 grams of sulfathalidine daily for the first 7 to 10 days.

RESULTS AND DISCUSSION

A summary of the sources and levels of carotene fed and the results obtained with the first 11 calves is shown in table 1. All of the calves except No. 1 failed to gain in weight and died at about seven days of age, regardless of the source of carotene or the amount fed. Post-mortem examinations indicated that death resulted from severe enteritis and/or septicemia and pneumonia. Insignificant amounts of carotene and vitamin A were found in the livers.

The blood-plasma levels of carotene and vitamin A for these calves are presented in figure 1. It will be noted from these data that without exception the plasma vitamin A did not show any appreciable increase. Although somewhat more variable, the plasma-carotene data seemed to indicate poor absorption of carotene. As shown in table 2, acute scours occurred in these calves shortly after birth and generally persisted until death. Since Adlersberg and Sobotka (1) demonstrated that humans with active sprue were unable to absorb fat-soluble vitamins, it seemed likely that the early onset of scours was at least partially responsible for the poor carotene absorption and low plasma vitamin A. Later it was found that even in older calves chronic scours decreases the absorption of carotene as exemplified in figure 2. A similar effect of scours on the absorption of vitamin A was also observed. Sutton and Kaeser (12) recently reported a simultaneous drop in blood plasma carotene and vitamin A in calves that had diarrhea, indicating poor absorption.

TABLE 1

Summary of experimental procedure and results obtained with calves developing scours shortly after birth

Number	Breed	Carotene		Birth wt.	Change in weight*	Age at death	Necropsy	Liver storage	
		Source	Daily intake					Carotene	Vitamin A
			mg./100lbs.	*lbs.*	*lbs.*	*Days*		*γ/gm.*	*γ/gm.*
1	H	Carotene in peanut oil	12	100	+5	19	Enteritis, septicemia, kidney degeneration, liver necrosis	1.0	0.0
2	G	Carotene in peanut oil	18	65	+2	9	Enteritis, pneumonia	0.0	0.0
3	H	Carotene in peanut oil	36	77	−3	11	Enteritis, septicemia	0.25	0.02
4	H	Carotene in lard	75	88	−10	4	Enteritis, pneumonia	0.38	0.02
5	J	Carotene in lard	165	66	−6	5	Enteritis, pneumonia	0.96	0.01
6	H	Alfalfa leaf meal	60	85	−5	3	Enteritis, pneumonia	0.04	0.0
7	G	Alfalfa leaf meal	60	64	−4	3	Enteritis, pneumonia	0.07	0.0
8	G	Alfalfa leaf meal	60	79	−10	9	Enteritis, pneumonia	0.10	0.01
9	H	Concentrate	5	92	0	8	Enteritis, septicemia	1.63	1.31
10	G	Concentrate	5	75	−4	5	Enteritis, pneumonia	0.64	0.0
11	H	Concentrate	8	98	−10	7	Enteritis, septicemia	0.12	0.28

* From birth until death.

While the exact cause of the early onset of diarrhea could not be ascertained, several factors were involved. These calves did not receive the natural protective immune bodies normally supplied in colostrum. Thus, favorable conditions existed for the development of infection and scours. The large amount of vitamin A, *per se*, which the newborn calf usually receives in colostrum may play an important rôle in the prevention of scours and infection. In fact, it was found (10) that calves died when the blood plasma vitamin A and carotene failed to increase following the ingestion of colostrum. The presence of both the immune bodies and the vitamin A in colostrum may be necessary to provide the greatest protection against scours and infection. Additional information on this problem is being accumulated by feeding skimmed colostrum, with and without large amounts of supplemental vitamin A.

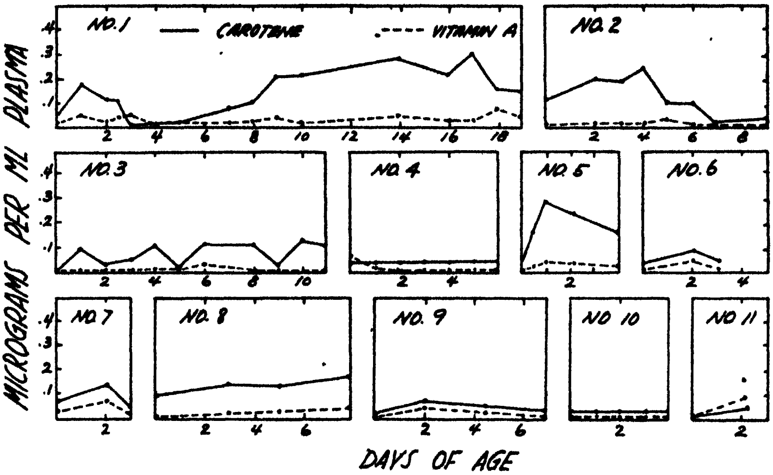

FIG. 1. Plasma carotene and vitamin A of the calves that developed scours shortly after birth.

The sources of carotene fed may have contributed to the poor absorption and utilization since difficulty was experienced in preparing a concentrated solution of carotene in oil suitable for the tests. It is believed that the large amounts of oil fed may have been responsible for scours in certain cases. Alfalfa leaf meal did not prove very satisfactory, probably due to the newborn calf's inability to efficiently digest fibrous feeds. Ward *et al* (13) found that carotene fed as a commercial concentrate in oil was more available to young calves than the carotene in alfalfa hay or other feedstuffs. This does not appear to be the case with older bovine, as Hauge *et al.* (5, 6) found that the mature dairy cow can utilize the carotene in alfalfa hay as readily as isolated carotene for the production of butterfat of high vitamin A value. Furthermore, Guilbert *et al.* (4) found that the minimum carotene requirements to prevent night blindness in cattle, sheep, and swine were

TABLE 2

The incidence and duration of scours of the calves listed in Table 1

No.	Breed	Carotene supplement	Daily intake	Day of age																			
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
			mg./100 lbs.																				
1	H	Carotene in peanut oil	12		+	+									+	+	+	+	+	+	+	+	Died
2	G	Carotene in peanut oil	18		+	+	+	+	+	+	+	+	Died										
3	H	Carotene in peanut oil	36						+	+	+	+	+	+	Died								
4	H	Carotene in lard	75	+	+	+	+	Died															
5	J	Carotene in lard	165		+	+	+	+	Died														
6	H	Alfalfa leaf meal	60	+	+	+	Died																
7	G	Alfalfa leaf meal	60	+	+	+	Died																
8	G	Alfalfa leaf meal	60		+	+		+		+	+	+	Died										
9	H	Concentrate	5			+	+	+	+	+	+	Died											
10	G	Concentrate	5			+	+	+	Died														
11	H	Concentrate	8			+	+	+	+	+	Died												

+ = Scours.

practically the same when the carotene was furnished by alfalfa or by crystalline carotene dissolved in cottonseed oil. Balance studies with newborn calves should contribute more evidence regarding the relative availability of carotene from different sources. The provitamin A activity of the several carotenoids present in alfalfa is as yet unsettled and may play an important rôle in this problem.

No information is available regarding the possible function of the tocopherols in carotene absorption and utilization by the newborn calf. It should be pointed out that every calf in this study received daily 50 milligrams of alpha-tocopherol in addition to that supplied by the alfalfa leaf meal or the cottonseed oil.

Little information regarding the ability of the newborn calf to utilize carotene was obtained from the foregoing calves and procedure, because of early infection and death. Therefore, further studies were undertaken. The commercial carotene concentrate, standardized to contain 33 mg. of carotene per gram, was used exclusively. Four to eight grams of sulfathalidine per day were administered orally to each calf during the first week. By following this procedure it was possible to reduce the incidence of early scours and to obtain a satisfactory survival rate as shown in tables 3 and 4.

The incidence and duration of scours in the individual calves receiving the commercial carotene concentrate and sulfathalidine are presented in table 3. It will be noted that without exception scours did not occur during the first five days following birth. It is likely that the more or less chronic scours observed in calf No. 12 was due to vitamin A deficiency as indicated by the low level of plasma vitamin A (figure 2). The presence of papilledema at 30 to 40 days of age was additional evidence of inadequate plasma vitamin A. Yellow, oil-like material was frequently observed in the feces from calf No. 15, due probably to the ingestion of excessive amounts of the carotene concentrate. This condition may have contributed to the manifestation of mild diarrhea by this calf throughout the experimental period.

As noted in table 4, four Holstein, one Jersey, and two Guernsey male calves receiving no colostrum were raised from birth to 60 days of age on a diet in which carotene was the only source of vitamin A. With the exception of calf No. 12, which received only 2 mg. of carotene per 100 lbs. of body weight, the calves made satisfactory growth gains and exhibited no symptoms of vitamin A deficiency.

The blood plasma carotene and vitamin A levels of these calves from birth to 60 days of age are shown in table 5. The data on calves number 12, 13, and 18 were selected as typical and presented in graphic form in figure 2. As shown by these data, the calves were able to absorb and utilize carotene when scours were prevented during the first week. Immediate conversion of carotene into vitamin A was efficient enough to cause a marked rise in the plasma level of vitamin A. At one day to two days of age the

TABLE 3

The incidence and duration of scours in the calves receiving sulfathalidine and the commercial carotene concentrate

No.	Breed	Carotene supplement	Daily intake	Day of Age																													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
			mg./100 lbs.																														
12	H	Concentrate	2						+	+	+		+	+	+	+		+		+	+	+			+			+		+			+
13	H	Concentrate	15																														
14	H	Concentrate	30																			+						+					
15	H	Concentrate	130						+		+	+	+	+		+	+		+	+		+		+	+			+	+			+	
16	J	Concentrate	66																														
17	G	Concentrate	40							+				+																+			
18	G	Concentrate	10											+	+	+	+					+	+	+	+			+	+	+	+		

+ = Scours.

TABLE 4

Summary of experimental procedure and results indicating carotene utilization

Number	Breed	Carotene		Birth Wt.	Weight gain*	% of normal gain	Age when killed	Necropsy	Liver storage	
		Source	Daily intake						Carotene	Vitamin A
			mg./100 lbs.	*lbs.*	*lbs.*		*days*		*γ/gm.*	*γ/gm.*
12	H	Concentrate	2	97	18	26	60	Papilledema at 30–40 days old	0.57	0.01
13	H	Concentrate	15	92	72	103	60	Normal	1.27	1.46
14	H	Concentrate	30	97	71	101	60	Normal	1.53	2.21
15	H	Concentrate	130	102	74	106	60	Normal		
16	J	Concentrate	66	48	41	100	60	Normal		
17	G	Concentrate	40	75	37	88	60	Normal	5.05	1.47
18	G	Concentrate	10	65	32	79	60	Normal	1.35	1.50

* From birth to 60 days of age.

TABLE 5

Carotene and vitamin A content of blood plasma of calves utilizing carotene from birth to 60 days of age

Days of age	No. 12		No. 13		No. 14		No. 15		No. 16		No. 17		No. 18	
	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A
	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.
Birth	0.01	0.03	0.02	0.02	0.01	0.01	0.02	0.03	0.05	0.01	0.09	0.02	0.06	0.03
1– 2	0.08	0.09	0.20	0.12	0.30	0.10	0.48	0.11	0.49	0.10	0.31	0.11	0.27	0.07
3– 7	0.11	0.09	0.25	0.12	0.70	0.15	1.23	0.18	0.75	0.16	0.52	0.09	0.50	0.11
8–14	0.09	0.05	0.29	0.15	0.80	0.16	0.92	0.25	1.51	0.31	0.56	0.07	0.49	0.13
15–21	0.08	0.04	0.30	0.10	0.83	0.30	0.94	0.18	1.80	0.32	0.74	0.08	0.45	0.10
22–28	0.09	0.03	0.28	0.08	1.05	0.28	0.70	0.06	1.46	0.25	0.64	0.05	0.33	0.06
29–35	0.15	0.07	0.30	0.10	0.90	0.20	1.14	0.21	1.23	0.23	0.60	0.04	0.35	0.05
36–42	0.20	0.10	0.26	0.10	0.88	0.18	1.24	0.27	0.78	0.18	0.64	0.06	0.51	0.07
43–49	0.12	0.08	0.30	0.13	0.92	0.20	1.11	0.19	1.25	0.21	0.76	0.07	0.68	0.11
50–60	0.10	0.06	0.30	0.11	0.85	0.18	1.08	0.21	1.32	0.25	0.96	0.07	0.51	0.12

plasma vitamin A levels were equal to or above the 0.1 microgram per ml. level which has been suggested (2) as the minimum below which deficiency symptoms occur. Additional evidence of the utilization of carotene was provided by the presence of appreciable amounts of vitamin A in the livers of the calves sacrificed at 60 days of age (table 4).

In view of the present paucity of knowledge regarding the complex factors involved in the physiological rôle of colostrum, experiments using the no-colostrum technique are not without reproach. While this technique is advantageous experimentally, caution should be practiced in drawing conclusions which may, of necessity, be revised as additional information is obtained.

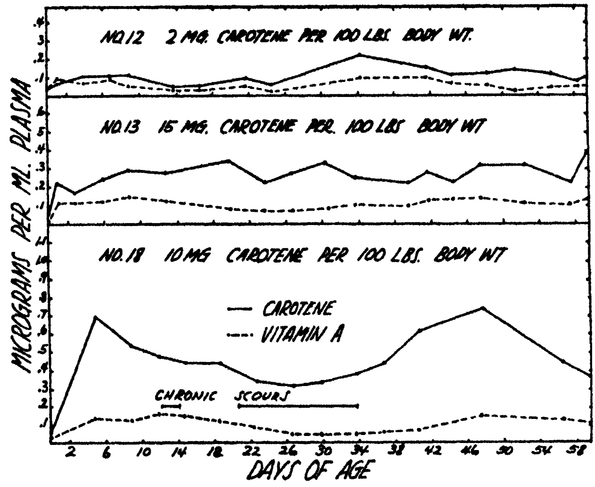

FIG. 2. Plasma carotene and vitamin A of three calves selected as typical of the calves utilizing carotene from birth to 60 days of age.

SUMMARY

Experiments were conducted with 18 newborn dairy calves to study carotene utilization. Holstein, Guernsey, and Jersey male calves were fed a skim milk–low carotene ration from birth without having received colostrum. Crystalline carotene in peanut oil or lard, alfalfa leaf meal and a commercial carotene concentrate were used as sources of carotene. Blood plasma levels and liver storage of carotene and vitamin A, growth and general well-being were used as criteria of carotene utilization.

The onset of infection and scours shortly after birth reduced the absorption and utilization of carotene regardless of the amounts fed.

Alfalfa leaf meal and laboratory preparations of crystalline carotene in peanut oil or lard were unsatisfactory as sources of carotene for the newborn calf.

Satisfactory prevention and control of scours was obtained in most cases by oral administration of 4 to 8 grams of sulfathalidine daily for the first week.

When scours were controlled, newborn calves were able to utilize the carotene in a commercial concentrate at a rate sufficient to provide for satisfactory growth and some storage in vitamin A in the liver.

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CLIPPING VS. GRAZING BY DAIRY HEIFERS AS MEANS OF ESTIMATING YIELD OF BLUEGRASS PASTURES[1, 2]

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In connection with a pasture experiment on the Experiment Station farm at Lexington, clippings were made at approximately two-week intervals throughout the grazing season from areas protected from grazing by wire cages. The estimated yield of total digestible nutrients (TDN) calculated from the amount of clippings obtained during five grazing seasons, 1940 to 1944 inclusive, is compared in this paper with that calculated from maintenance requirements and gain or loss in weight of dairy heifers grazing on the pasture.

EXPERIMENTAL CONDITIONS

The experiment pasture, consisting principally of Kentucky bluegrass (*Poa pratensis*) and white clover (*Trifolium repens*), was more than 25 years old. Alfalfa, red clover, alsike clover, sweet clover and Korean, Kobe and common lespedeza made up small portions of the flora. The pasture was comparatively free from weeds. Ragweed, dandelion, horsenettle, sourdock, buckhorn and yarrow were present, but in such small amounts as to contribute little to the herbage. The pasture had an area of 11.6 acres and its length was about twice its width.

No fertilizer was added to the pasture during this experiment except the droppings of the heifers. From time to time the droppings were scattered with a drag made from automobile tire rims chained together. Twice each year the pasture was clipped with a mowing machine, adjusted to cut about six inches above the soil level.

A total of 71 Jersey and 45 Holstein heifers were used in the five years

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by the permission of Thomas P. Cooper, Director of the Kentucky Agricultural Experiment Station in connection with Bankhead-Jones Project No. 6.

[2] The project was conducted cooperatively by the Dairy Section and the Agronomy Department. Yields of dry matter from which TDN in the harvested herbage was calculated were obtained by the Agronomy Department and are used with the permission of that department.

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of the experiment reported. The average age of the heifers when put in the pasture was 15 months and 2 days, and the average weight was 575 pounds. Starting ages ranged from 6 months and 13 days to 23 months, though 76 per cent of the heifers were between 10 and 12 months. Starting weights ranged from 229 to 1024 pounds, but 70 per cent of the heifers weighed between 400 and 750 pounds. Only heifers that appeared healthy were used.

Grazing was started as early in the spring and continued as late in the fall as seemed consistent with weather conditions and growth of grass. Heifers were conditioned to grazing by being on blue grass pasture about two weeks before experimental grazing was begun. During this period and while they were on the experimental pastures they had no supplemental feed except salt and sometimes bonemeal. The number of heifers on the pasture was varied in accordance with the judgment of the investigators and was based on the condition of the pasture, the weather, and the amount of weight gained or lost by the heifers during the preceding two-week period. An effort was made to keep the plots pastured up to their full capacity but not overgrazed. Grazing to full capacity was not possible during the period of most rapid growth of grass, usually May and June, when there were not enough heifers available. The maximum number of heifers on the 11.6 acres at one time was 22 and the minimum 4. The maximum weight of heifers on the pasture at one time was 15,929 pounds and the minimum 2239 pounds. Before being placed on pasture and at two-week intervals thereafter, each heifer was weighed on three consecutive days to obtain her average weight at that time. In case a heifer was in heat on one of the three weigh days the weight for that day was not used in determining the average weight because weights during estrus averaged somewhat below normal.

The total digestible nutrients obtained by the heifers from the pastures was calculated by the method of Morrison and Ely (11). By this method, 3.53 pounds of TDN were allowed per pound of gain and 2.73 pounds of TDN subtracted per pound loss in weight, as suggested by Knott, Hodgson and Ellington (9) and recommended by the Joint Pasture Committee (8). To calculate requirements the minimum amount of TDN advised by Morrison (10) for maintenance of dairy cows was applied to the average weight of all heifers on the pasture during the five seasons of this study. As Morrison's data are based only on cows weighing 700 pounds or more, amounts recommended by him for cows of various weights were plotted and the curve extended to obtain requirements of animals weighing 600 pounds or less.

TDN in clippings were calculated from yields of herbage harvested from 12 caged areas in representative parts of the pasture. Seventy-two per cent of the oven-dry matter was considered to be digestible (8). Each area contained 41.8 square feet. The areas were protected from grazing by wire cages and harvested on approximately the dates when the heifers were weighed. Harvesting was done with a lawnmower set to cut the grass about one inch above the ground and the clippings were collected in a grass catcher attached to the mower. After each mowing, the cages were moved to new locations where the herbage was evenly grazed. The lawnmower was run over each area before the cage was placed.

RESULTS

In 1940, heifers were turned on the pasture April 12. Chart A of figure 1 shows the average daily yield of TDN throughout the season as calculated from clippings and from heifers weights. Chart B gives the cumulative total of TDN throughout the pasture season as calculated by the two methods. Chart C shows mean temperature from the records of the Lexington weather bureau station, averaged by 5-day periods, *e.g.*, the average

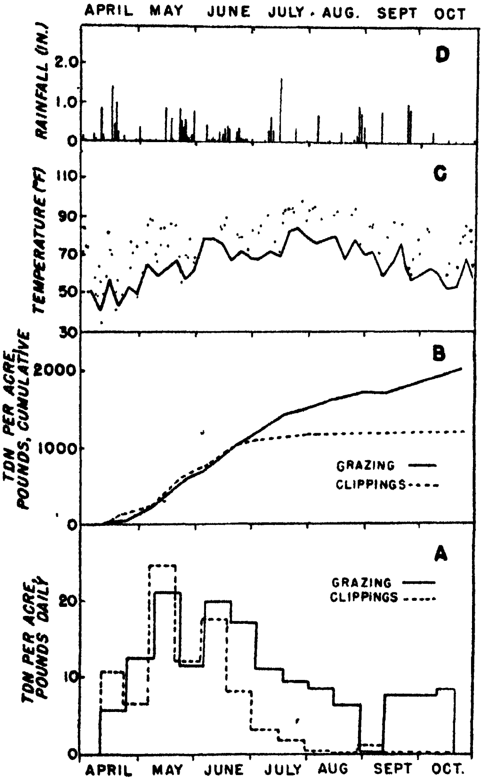

FIG. 1. TDN yields and weather conditions during the 1940 grazing season. A, daily production of TDN calculated from herbage yields and grazing data. B, accumulated production of TDN calculated from herbage yields and grazing data. C, maximum and average temperatures by 5-day periods. D, daily rainfall.

of the mean temperatures May 3 to 7 inclusive is plotted on May 5. Dots show the maximum temperature recorded each day. Chart D shows the inches of daily rainfall recorded by the Lexington weather bureau station.

The yield of TDN for the 1940 season calculated from clipping data was 1227 pounds or 59 per cent of that from grazing data (table 2 and fig. 1, B). Daily yields, as calculated from both clipping and grazing data (fig. 1, A) reached a high peak during the middle two weeks of May and a secondary peak in June. Doubtless both peaks were caused primarily by the high rainfall in mid-April and late May (fig. 1, D). Following the middle of June, the TDN estimated from the clippings fell rapidly to a very low level and remained low during the rest of the season, probably because of relatively low rainfall. Yields of TDN calculated from grazing data, on the contrary, declined rather moderately in late June and were substantial after July 1, except during early September. The estimates of accumulated

TABLE 1

Summary of data on rainfall, temperature, and TDN calculated from herbage yields and grazing data

(Yields of TDN are stated as pounds per acre)

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Total or ave.
1940									
Rainfall—inches	4.8	5.4	4.0	3.4	3.3	2.6	0.6		24.1
Mean temp.—°F.	51.6	59.8	73.4	76.4	76.7	66.7	65.2		67.1
TDN from clipping	181	518	398	97	13	17	3		1227
TDN from grazing	138	504	516	358	222	135	204		2077
1941									
Rainfall—inches	2.2	1.7	4.2	7.3	1.9	0.7	4.2		22.2
Mean temp.—°F.	59.1	68.1	74.6	78.1	76.9	73.6	63.0		70.5
TDN from clipping	146	213	195	217	68	41	9		889
TDN from grazing	208	506	405	214	316	183	17		1849
1942									
Rainfall—inches	3.1	3.4	5.5	8.2	7.3	3.0	3.5		34.0
Mean temp.—°F.	57.6	64.6	74.4	78.2	75.5	67.4	58.0		68.0
TDN from clipping	251	310	80	198	333	217	87		1476
TDN from grazing	263	658	278	299	365	481	271		2615
1943									
Rainfall—inches	4.0	6.7	3.1	7.7	1.1	1.9	1.6		26.1
Mean temp.—°F.	50.8	66.4	78.0	77.6	78.5	67.4	56.9		67.9
TDN from clipping	141	1009	543	138	203	39	3		2076
TDN from grazing	62	507	633	397	250	335	96		2280
1944									
Rainfall—inches	4.2	3.5	1.6	2.8	6.2	2.4	1.9	1.6	22.6
Mean temp.—°F.	53.8	70.9	77.5	77.7	79.9	67.4	56.1	43.8	69.0
TDN from clipping	254	758	168	34	322	496	174	12	2218
TDN from grazing	242	717	572	92	164	466	378	72	2703
5-year average									
Rainfall—inches	3.7	4.1	3.5	5.9	4.0	2.1	2.4		25.7
Mean temp.—°F.	54.5	65.9	75.6	77.6	77.1	68.3	59.8		68.3
TDN from clipping	195	562	277	137	188	162	55	2	1578
TDN from grazing	183	578	481	272	263	320	193	14	2305

pasture production in 1940 by the two methods parallel each other quite closely until late in June after which yields calculated from clippings were substantially lower than those calculated from grazing data (fig. 1, B).

During the 1941 season the total yield of TDN calculated from clipping data was 889 pounds or 48 per cent of that calculated from grazing (table 2 and fig. 2, B). The highest yield from clipping was in late April and early May. Lesser peaks of yield occurred in late June and July in response doubtless to the above-average rainfall in those months. Yields of TDN estimated from grazing data in April were much higher than those from clippings. Peak yields occurred in late May and early June, August and September (fig. 2, A). Yields of TDN as calculated by both methods accumulated at similar rates until late June. Thereafter those obtained from grazing continued to increase while those from clippings increased but

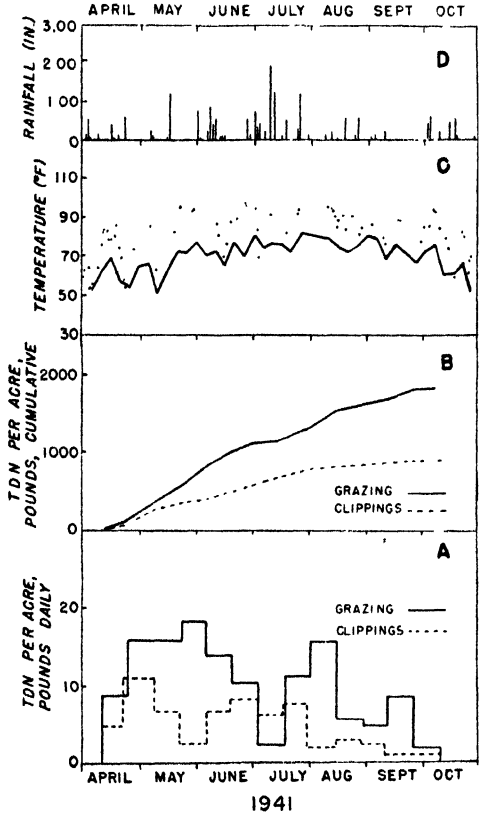

FIG. 2. TDN yields and weather conditions during the 1941 grazing season. A, daily production of TDN calculated from herbage yields and grazing data. B, accumulated production of TDN calculated from herbage yields and grazing data. C, maximum and average temperatures by 5-day periods. D, daily rainfall.

slightly. Doubtless the relatively low yields of TDN obtained in 1941 are correlated with the somewhat deficient rainfall of the season and the relatively high temperature (table 1).

The estimated yields of TDN from clipping and grazing in 1942 had similar trends throughout the season, although that calculated from clipping data (1476 pounds) was only 56 per cent of that calculated from grazing (table 2 and fig. 3, B). Yields from clipping were high in April and somewhat higher in early May. They dropped rapidly until mid-June and then rose to their highest figure of the year in August. They dropped again slightly in mid-August and after a slight rise in September were low during the rest of the season. Yields calculated from the grazing data reached a much higher peak in May than that from clipping, dropped to a low point in mid-June and then steadily increased to a secondary high point in late September. Yields of herbage as calculated from grazing and clipping accumulated at somewhat similar rates throughout the whole season (fig. 3, B). Rainfall in 1942 was higher than in any of the other seasons in this 5-year period (table 1). It was especially high in July and August, resulting in much growth of herbage from July to October. Monthly mean temperatures during the 1942 season were all very close to the 5-year average (table 1). The maximum temperature recorded during the grazing season was 98 degrees F. and temperatures of 90 degrees F. or higher were recorded on 42 days.

TABLE 2

TDN calculated from herbage yields and grazing data

Year	No. of days in each season	TDN from clipping		TDN from grazing		Clipping yield on basis of grazing yield as 100
		Total per acre	Per acre per day	Total per acre	Per acre per day	
		pounds	*pounds*	*pounds*	*pounds*	
1940	196	1227	6.26	2077	10.60	59.1
1941	182	889	4.88	1849	10.16	48.1
1942	210	1476	7.03	2615	12.45	56.4
1943	168	2076	12.36	2280	13.57	91.1
1944	210	2218	10.56	2703	12.87	82.1
Ave.	193.2	1578	8.16	2305	11.93	68.5

Total yields of TDN in 1943 estimated by the two methods (table 2 and fig. 2, B) were approximately equal, those calculated from clipping being only 9 per cent less than grazing. The slope of the curve expressing yield from clipping, however, was much steeper during the first two months and less steep during the last four months of the grazing season than that based upon grazing data. In the early part of the 1943 grazing season the TDN yield estimated from clipping reaching the highest point during this 5-year period (fig. 4, A). It averaged nearly 38 pounds daily per acre from May 7 to May 21. During the 53 days from April 26 to June 18 it averaged slightly over 30 pounds daily. It then dropped to a comparatively low level and remained rather low for the rest of the season. The estimated yield from the grazing data did not reach nearly so high a level as did that from the clipping but remained much more constant throughout the season. The high yield occurred late in May and remained fairly constant until mid-July. After four weeks at a low level it again rose to an average of more than 10 pounds of TDN daily and remained essentially constant during the rest of the season. Doubtless the very high yields of TDN produced before mid-July were correlated with the very high rainfall of 21 inches that fell between April 1 and August 1 (table 1). After August 1, there were only two dates on which more than one-half inch of rain was recorded.

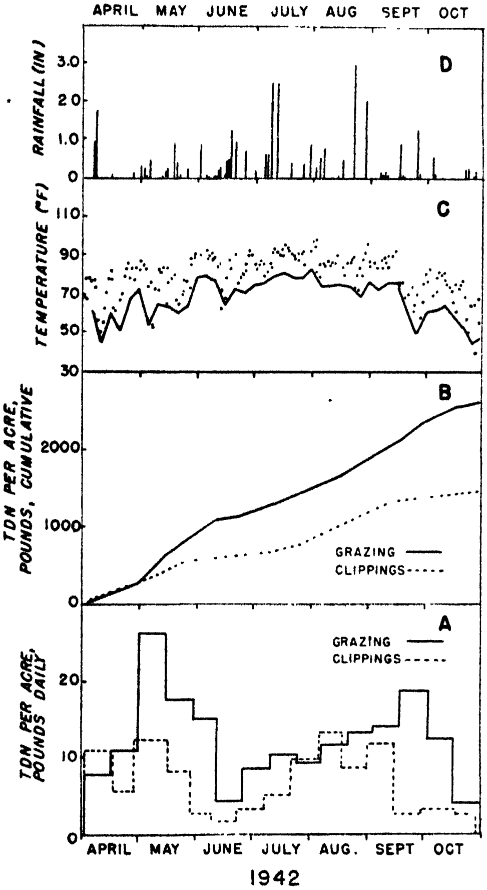

FIG. 3. TDN yields and weather conditions during the 1942 grazing season. A, daily production of TDN calculated from herbage yields and grazing data. B, accumulated production of TDN calculated from herbage yields and grazing data. C, maximum and average temperatures by 5-day periods. D, daily rainfall.

Monthly mean temperatures were not greatly different from the 5-year average but an unusual maximum temperature of 102 degrees F. was recorded in August and 12 of the 22 days when the temperature reached 95 degrees F. or higher were in August. Temperatures of 90 degrees F. or higher were recorded on 56 days in 1943 (fig. 4, C).

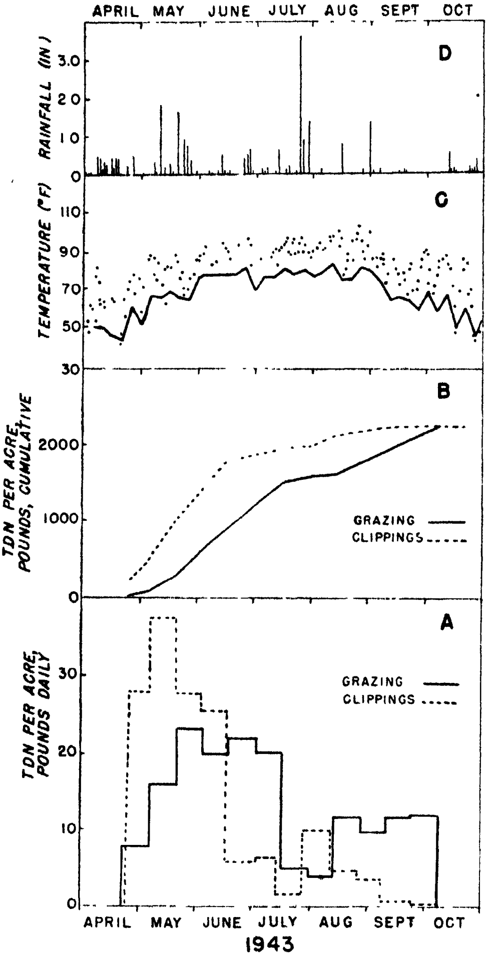

FIG. 4. TDN yields and weather conditions during the 1943 grazing season. A, daily production of TDN calculated from herbage yields and grazing data. B, accumulated production of TDN calculated from herbage yields and grazing data. C, maximum and average temperatures by 5-day periods. D, daily rainfall.

Total yield of TDN in 1944 estimated by the grazing method exceeded that obtained from clippings by 485 pounds per acre, or about 18 per cent. Accumulated yields by both methods were similar throughout the season of 1944 (table 2 and fig. 5, B). Two quite high peaks of production with a period of very low production between them are shown by both methods of measurement (fig. 5, A). High production of TDN, as calculated from clipping, occurred early in May. Thereafter there was a decline in production until late in June. Production again rose rapidly from early August to early September and then dropped steadily until the end of the season. Highest production as estimated from grazing also occurred in early May and production remained high until about June 1. Low production occurred in July and another period of high production late in September.

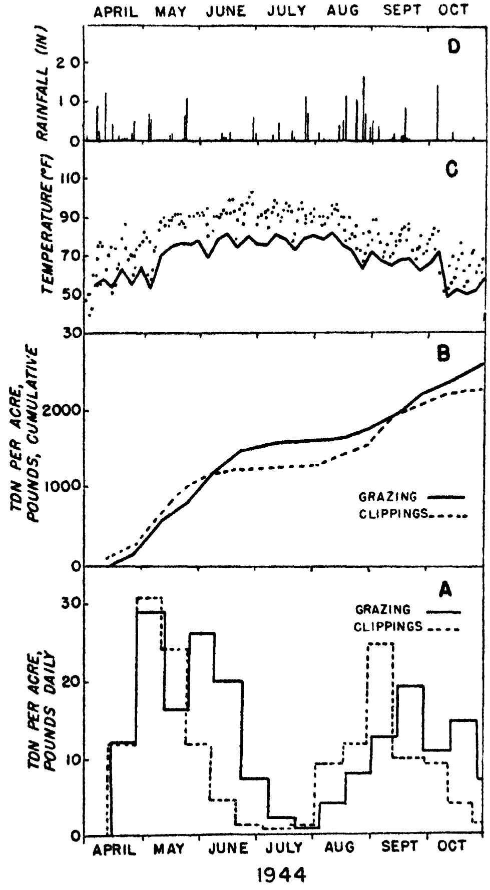

FIG. 5. TDN yields and weather conditions during the 1944 grazing season. A, daily production of TDN calculated from herbage yields and grazing data. B, accumulated production of TDN calculated from herbage yields and grazing data. C, maximum and average temperatures by 5-day periods. D, daily rainfall.

The curves of chart B, figure 5, show that the accumulated yields of TDN in 1944 as calculated by the two methods paralleled each other rather closely throughout the season. Doubtless the patterns of TDN yield curves in charts A and B were correlated with the good rainfall during April and May, the drouth in June and July, and the large amount of rainfall in August. Somewhat higher than average temperatures during June, July and August may have contributed to the low yields produced between mid-June and mid-August. The highest temperature of the season was 103 degrees F. on June 28. There were 66 days on which temperatures of 90 degrees F. or higher were recorded, 22 of these being in June and 21 in July.

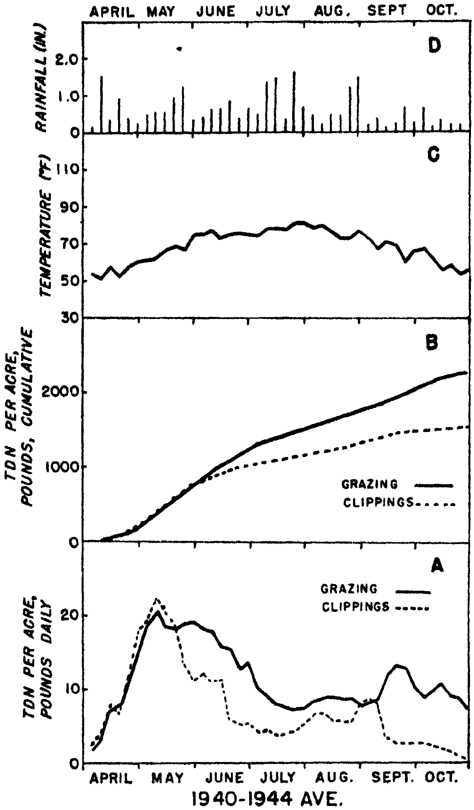

FIG. 6. Average TDN yield and weather conditions 1940–1944. A, average daily production of TDN calculated from herbage yields and grazing data. B, average accumulated production of TDN calculated from herbage yields and grazing data. C, average temperature by 5-day periods. D, average rainfall by 5-day periods.

A 5-day moving average of calculated TDN yields produced throughout the season during the 5-year period shows by both methods of calculation a peak in production on about May 11 (fig. 6, A). The yield calculated from clipping was slightly higher than that from grazing at that date, but about 10 days later the yield calculated from grazing became higher and remained in that relative position during the rest of the season except during a few days in early September. The 5-day average cumulative yield of TDN calculated from clipping was slightly higher than that calculated from the data on grazing by heifers up to June 1, but after that date the grazing data indicate the higher yield (fig. 6, A). The average total annual yield estimated from clipping was 1578 pounds per acre, or about 68 per cent of that from grazing (table 2). For comparison the average temperatures for the 5 grazing seasons are given by 5-day periods in table 1 and fig. 6, C, and the average daily rainfall is shown in fig. 6, D. Monthly and seasonal rainfall and monthly and seasonal mean temperature are shown on table 1.

DISCUSSION

Under the conditions of this experiment with Kentucky bluegrass pasture, the average yield of TDN calculated from grazing data agreed fairly well with that calculated from clipping the herbage during the spring. During the remainder of the season the yields calculated from grazing data were larger than those calculated from clippings. Yields of TDN calculated from clippings in the 5 years of this experiment ranged from 48.1 to 91.1 per cent (average 68.5 per cent) as large as those from grazing. Considerably less variation occurred from season to season in the yields estimated from grazing data than in the estimates from clippings. By both methods of measurement highest yields were obtained in 1944 and lowest yields in 1941. Yield in 1940 was next to the lowest. The order of yields obtained in 1942 and 1943 is reversed by the two methods of calculation.

Results of this experiment are not in agreement with those obtained by several other investigators (1, 3, 4, 5, 6, 7,) who found that the yield of TDN estimated from clippings usually exceeded that calculated from grazing. Robinson, Pierre and Ackerman (12) reported that grazing indicated higher yields in four out of five years. However, these reports dealt with many kinds of pasture grasses and legumes and different grasses and legumes are adapted to different techniques in clipping and pasturing (2, 8). In the case of bluegrass, a small number of animals can be placed on the pasture as soon as the grass starts growing actively in the spring and the number can be increased as the growth indicates that a larger number can be supported. Furthermore, bluegrass can be grazed rather close during the entire season. On the other hand, some pasture crops must be permitted to attain considerable growth before pasturing and many cannot be grazed as close throughout the season. Such pasture crops are not likely to be fully utilized by grazing, and it would appear, therefore, that yields of TDN obtained by clippings would be higher than those calculated from grazing data.

In calculating pasture yield in terms of TDN in this experiment the amount of TDN allowed for maintenance plus gain was slightly higher than the amount allowed by some investigators (12) when gains of heifers were about one pound daily; but when gains of two or more pounds daily were made by heifers, the amounts of TDN allowed for maintenance plus gain in this experiment were considerably higher than allowed by the method used by Robinson *et al.* (12). In many of the comparisons of methods of measuring pasture yields by other investigators milking cows have been used as experimental animals. Usually the milking cows have had supplementary feeding, which presumably would cause them to eat less grass than animals which receive no feed other than pasture.

As might be expected, the curves of grazing yield obtained in this experiment show increases and decreases in calculated yield of TDN from grazing to occur one to two weeks later than from clipping. Grazing yields are not quite as high as clipping yields in the spring when the growth of grass is most rapid, but the heifers were able to utilize the excess grass a little later when the growth of grass had slowed down. Doubtless the lag might have been cut down somewhat if more heifers had been available for the experiment, but even so, the pasture carried an average of almost two heifers per acre during the periods of most rapid growth of grass. Later in the season the trends of curves of the 5-year average yields from clipping and from grazing follow each other quite closely, but the curve for grazing is somewhat higher and changes about two weeks after the change indicated by clipping.

The method of obtaining clippings used in this study, in which the cages were moved at two-week intervals to rather close-grazed areas, may give somewhat low results especially during periods of slow growth. Especially during drouths there were times when many of the leaves of grass on the new locations for the cages were below the level at which the lawnmower cuts. The clippings obtained at the end of the following two-week period, therefore, may have been somewhat less than the actual growth during the period.

In estimating the yield of pasture, or its value as a source of feed for cattle, it is axiomatic that the effective yield of pasturage can be measured only by grazing. However, when it is not practicable to measure yield of Kentucky bluegrass pasture by animals, clipping protected areas as done in this experiment is a reasonably accurate method for determining relative yield.

SUMMARY

Yield of a Kentucky bluegrass pasture in terms of TDN per acre was calculated from clipping with a lawnmower and from grazing with dairy heifers during five seasons, 1940–1944 inclusive, the grazing season ranged from 168 to 210 days in length.

Yield calculated from grazing was higher than that calculated from clipping in each of the five seasons. The calculations from clipping were 50.1,

48.1, 56.4, 91.1, and 82.1 per cent of those from grazing in 1940, 1941, 1942, 1943 and 1944, respectively. The average calculated yield from clipping was 68.5 per cent of that from grazing.

The maximum, minimum and average yields of TDN calculated from clipping were 2218, 889 and 1578 pounds per acre respectively, and from grazing 2615, 1849 and 2305 pounds.

The average daily amount of TDN furnished by the pasture for an entire season ranged from 4.88 pounds to 12.36 pounds per acre as calculated from clipping, and from 10.16 pounds to 13.59 pounds as calculated from grazing.

The trend of the curves representing calculated yield obtained by clipping and grazing with heifers were in general quite similar, although a pronounced change in yield usually occurred in the grazing curve two to three weeks later than in the clipping curve.

The data obtained in this experiment indicate that the clipping method of measurement may be used in a preliminary way to evaluate variables in experimental management of Kentucky Bluegrass pastures.

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A SYNTHETIC PABULUM FOR THE PRESERVATION OF BULL SEMEN[1]

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The yolk-buffer pabulum for the preservation of bull sperm (1, 2) was developed to meet practical conditions of artificial insemination. An attempt was made to provide a sufficiently favorable environment to maintain the virility of the sperm for periods up to 96 hours or more. In the first full report (2) data were available to show that cows could be bred with semen 100 hours old. From these data it seemed that it would be possible to have on hand a usable semen sample from any given bull every day of the month. In actual practice this goal has not been attained.

Various attempts have since been made to improve yolk-buffer as a storage pabulum for bull sperm. Knoop (3) presented evidence to show that the addition of small amounts of gelatin added to yolk buffer lengthened the interval that bull sperm could be stored. Later Knoop and Krauss (4) reported that diluents containing 1.0 per cent glycine, or 1 per cent *l*-proline and without gelatin were superior to egg-yolk-buffer-gelatin, for sperm survival. The inference was that these amino acids were the active ingredients responsible for the gelatin stimulation. Salisbury, Fuller and Willett (5) substituted sodium citrate for the dibasic sodium phosphate of the Phillips formula and obtained better visibility of individual spermatozoa in the resulting mixture since the citrate caused a noticeable dispersion of the fat globules of the egg yolk.

Phillips and Lardy (2) in 1940 and again in 1942 (6) called attention to the resistance of yolk-buffered bull semen to cold shock. This observation was confirmed and extended by Lasley, Easley and Bogart in 1942 (6). Mayer and Lasley (7) reported the preparation of a ''resistance factor'' in egg-yolk which was an acetone, alcohol ether-insoluble crystal-like substance giving a positive ninhydrin reaction. The authors report this substance to be active in a ratio of 1:250. Lardy and Phillips (8) presented evidence in 1942 to show that the addition to yolk-buffered bull semen of glucose, glucose and dibasic sodium phosphate, or dibasic-sodium-phosphate-glucose and adenylic acid prolonged active motility over that obtained with yolk-buffered semen only. By far the greatest stimulation was obtained in the combination wherein glucose and adenylic acid were used. This led

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to the suggestion that the loss of motility may be the result of loss of dialyzable coenzymes.

We have previously pointed out that bull sperm obtained their energy from two sources, phospholipids (acetone-insoluble proportion of egg yolk, or soybean lecithin) and glucose, that their energy was obtained by one of two mechanisms, oxidation or glycolysis (9, 10) and when oxidation and glycolysis are inhibited, a limited degree of motility is supported by some other means (probably reserve high energy phosphate) (11). Salisbury and Vandemark (12) presented evidence to show that small amounts of glucose added to egg yolk citrate increased the livability and lactic acid production of bovine semen. These authors state that "the glucose loss was not directly related to the livability of the spermatozoa unless that sugar was glycolyzed to lactic acid." This confirms the report of Lardy and Phillips in 1941 (9) when they showed that "added sugars maintained motility only if they could be catabolized to lactic acid by the spermatozoa."

Despite these data field inseminations show that yolk-buffered semen (alone and in its various modified forms) continues to be used before the end of 72 hours. The opinion prevails that the "mine-run" sample of yolk-buffered semen is not dependable beyond this period. While this opinion is not fully supported by our laboratory data it is recognized that there is a progressive loss of metabolic activity, motility and vitality which results in lowered breeding performance somewhere between the 60th and 125th hours of storage. The success and extent of artificial insemination as an instrument for cattle improvement depends upon how successfully bull sperm for breeding purposes can be stored and preserved.

The present report deals with a considerable number of observations pertinent to the storage of bovine semen and which have made possible the development of a synthetic pabulum for bovine semen storage.

EXPERIMENTAL

The experiments herein reported were designed to study the effects of certain proteins, lipids and carbohydrates or their metabolic intermediates upon bull spermatozoa storage. Further, the effects of certain gums or bactericidal agents were also investigated. These constituents were studied singly or in various combinations, and their ability to environ sperm was measured against control aliquots of bull sperm stored as yolk-buffered semen. In all cases the storage interval was used as the measure of adequacy of the storage pabulum and the end point used was the time when a clear-cut demonstration was evident in the superiority or inferiority of the storage medium.

The samples were rated on the basis of their motility. A value of 5+ was given the best samples and 1+ for the poor samples. The rating was made when there was no longer distinctly active motility and when approximately ¾ of the sperm were immobilized. Because of the variation between experiments each experiment carried its own yolk-buffered sample as a standard of comparison. The sperm samples were checked daily in duplicate under the microscope at 30° C. and motility ratings made and recorded. When a single sample reached a 1+ motility on duplicate observations the entire experiment was terminated. In order to conserve space summarizing tables were made which give only the experiment number, the terminal motility rating for each experimental treatment and the time required to reach the motility ratings. Thus by noting the total length of the storage period in hours under any given experimental treatment a comparison of the effect of the environmental pabulum upon motility in storage could be made.

Unless otherwise stated, buffer concentrations were 0.15 M. This conforms to our previously established optimal molarity. The buffer salts employed were mono-basic potassium phosphate and dibasic sodium phosphate in amounts sufficient to give a final pH of 6.8–7.0 for the respective mixture. The other constituents were varied as indicated. All samples were stored in the refrigerator at 4–5° C. The lipositol used in these experiments was made according to the method outlined by Woolley (14). Lecithin was purified by two extractions each of alcohol and acetone with constant stirring at room temperature. The lipoid fraction must be used fresh or stored under conditions which eliminate hydrolysis. Slightly hydrolyzed lecithin or lipositol gave very inferior results. Both preparations were used after 2 to 3 months storage in the refrigerator at 5° C.

RESULTS

The data presented in table 1 were selected from different experiments and are typical of the results obtained. In all cases the experiments were repeated two or more times and many were checked by as many as 10 repeat experiments with different semen samples from the same or different bulls. In these experiments the amino acid arginine, or the protein of blood fibrin was without stimulatory effect upon storage. Inspection of the data indicate that purified lecithin or lipositol preparations from crude soybean lecithin when used to replace the yolk component of yolk-buffer in the presence of 0.04 M glucose, or a mixture of galactose (0.02 M) and glucose (0.04 M) was equal to and in most cases superior to yolk-buffer as a storage medium for bull sperm. Glucose added to yolk buffer improved the storage medium but not uniformly so. Favorable concentrations of the glucose additions to yolk buffer were found to be 0.02 to 0.04 M. Individual components of lipositol, such as tartaric acid and with the exception of inositol were without stimulatory effect. Inositol when properly buffered and with added glucose and galactose promoted excellent motility for prolonged periods. The optimum concentrations of inositol were found to be 0.05 to 0.01 M solutions.

In an attempt to determine the action of certain sperm inhibitors it was observed by Phillips and Lardy (13) that certain gums noticeably reduced the inhibitory action. It seemed from this that the use of certain of the commercial gums might be beneficial to sperm storage. Table 2 indicates the effect of these gums upon sperm survival. Each of these experiments was repeated from two to four times. It is evident that gum damar and guaiac were definitely toxic while gum arabic, acacia, mesquite, ghatti and tragacanth were not harmful. Each of these gums was helpful in that they formed sufficient body to prevent the settling out of sperm upon standing in storage. The concentrations which were found to be desirable and which were used in the later experiments were acacia 2 to 3 per cent, mesquite, arabic and ghatti 2 per cent, and tragacanth 0.5 to 1.0 per cent.

TABLE 1

Data of selected experiments which show the influence of certain factors upon bull spermatozoa preservation

Treatment of semen	Data from experiment No.	Semen evaluation after storage	
		Rating	Hours stored
YB only (control)	1	1+	96
YB + arginine (1½ mg. in 5 cc.)	1	1+	96
YB only (control)	2	1+	96
YB + blood fibrin (0.5%)	2	1+	96
YB only (control)	3	1+	96
Lecithin prep. + 0.04 M glucose (buffered pH 7.8)	3	2+	96
YB only	5	1+	168
YB only (control)	8	fm*	96
YB + 0.04 M glucose	5	2+	168
YB + 0.04 M glucose	8	fm	96
YB + 0.04 M galactose	8	fm	96
Lecithin prep. + 0.04 M glucose	8	1+	96
Lecithin prep. + 0.04 M glucose + 0.02 M galactose	8	2+	96
Lecithin prep. + 0.04 M galactose	20	2+	96
Lipositol + 0.04 M glucose + 0.04 M galactose	20	2+	96
Galactose + glucose + tartaric acid	8	fm	96
Pure inositol + galactose + glucose + tartaric acid	8	1+	96
YB only (control)	8	fm	96
Inositol + 0.04 M glucose	8	1+	96
YB only (control)	17	2+	96
Tragacanth, lecithin-buffer-glucose	17	2+	96
Buffer-glucose-lecithin	17	1+	96
Gum-buffer-glucose-lipositol	26	1+	168
Gum-buffer-glucose-lecithin	26	1+	168

* fm = few motile.

Since experiment 8 indicated that tartaric acid was not toxic to sperm an experiment was made to determine if other dicarboxylic acids were stimulatory to sperm in storage. The basic formula used as a control in this experiment was the same as that indicated in table 2.

TABLE 2

Data on effect of certain gums upon bull sperm storage

Gum†	Data from experiment No.	Semen evaluation after storage	
		Rating	Hours stored
Buffer solution only	16	1+	48
Buffer-tragacanth	16	fm*	48
Buffer-tragacanth-lecithin	17	1+	144
YB (control)	17	1+	144
Tragacanth	21	1+	168
Mesquite	21	1+	168
Arabic	21	2+	168
Damar	21	0	24
Guaiac	21	0	24
Ghatti	21	1–2	168
Acacia	21	1+	168
YB + 0.04 M glucose	21	1+	168

* fm = few motile.

† Basic formula used to test action of gums.

Glucose	0.6%
Galactose	0.2%
KH_2PO_4	0.2%
$Na_2HPO_4 12H_2O$	1.8%
Lipositol	1.0%

It is evident that the dicarboxylic acids tested are not capable of sustaining motility in storage. The slight beneficial effect of adipic acid was not confirmed in subsequent tests. Tartaric acid, pimelic and adipic acid additions to the base alone gave 5+, 5+ and 3+ motility, respectively, at 48 hours.

Because of the flagrant growth of bacteria in some of the combinations used in these experiments and whereas we had found in 1943 (13) that cer-

TABLE 3

Data on the effect of certain dicarboxylic acids upon bull spermatozoa survival

Acid added—basic formula as previously used	Semen evaluation after storage	
	Rating	Hours stored
Base + lipositol + glutamic acid, 0.01 M	1+	24
Base + aspartic acid, 0.01 M	1+ (many club tailed)	24
Base + tartaric acid, 0.01 M	1+*	72
Base + succinic acid, 0.01 M	1+*	72
Base + malic acid, 0.01 M	1+	72
Base + citric acid, 0.01 M	1+	72
Base + glutaric acid, 0.01 M	1+*	48
Base + adipic acid, 0.01 M	3+	48
Base + pimelic acid, 0.01 M	3+	48
Base + lipositol control	4+	72
Base + lipositol + adipic acid, 0.01 M	5+	72

* Heavy bacterial growth observed.

tain antibiotics including penicillin were not harmful to bull sperm motility, a series of experiments were then made to determine the effect of certain sulfonamides, penicillin, and streptomycin upon sperm storage. The base used in these experiments was that previously indicated plus three per cent gum acacia. The results are shown in table 4. It is apparent that sulfapyridine was definitely toxic and that certain of the sulfonamides and streptomycin did not impair sperm motility in storage. Sulfasuxidine, sulfathalidine, sulfaguanidine and streptomycin appeared to favorably influence the maintenance of motility. This action was particularly noticeable in some samples of semen. Apparently gross bacterial contamination was sufficient to seriously curtail survival of sperm in these cases and the antibiotic action of these drugs was therefore beneficial.

TABLE 4

Data on the effect of antibiotics on sperm storage

Drug used	Data from experiment No.	Semen evaluation after storage			
		Rating	Hours stored	Rating*	Hours stored
Base only (control)	28	3	96	1 +	168
Base + sulfathiazole (30 mg.%)	28	3 +	96	vfm	168
Base + sulfadiazine (30 mg.%)	28	4 +	96	vfm	168
Base + sulfapyridine (30 mg.%)	28	1 +	96	vfm	168
Base + sulfaguanidine (30 mg.%)	28	3 +	96	2	168
Base + sulfasuxidine (30 mg.%)	28	4 +	96	1	168
Base + sulfathalidine (30 mg.%)	28	4 +	96	2	168
Base + sulfathiazole (30 mg.%)	29	3 +	120	vfm	144
Base + sulfathiazole + Lasley prep.	29	3 +	120	fm	144
Base + Lasley prep.	29	4	120	fm	144
Base + streptomycin	29	2	168	1 +	192
Base + sulfasuxidine	29	2	168	1 +	192
Base + sulfathalidine	29	1	168	1 +	192
Base only (control)	29	1	168	fm	192

* fm = few motile.
vfm = very few motile.

On the basis of the foregoing results a synthetic bull sperm pabulum was developed for the purpose of environing them for storage purposes. This pabulum was made up as indicated below. It possesses an outstanding advantage in that each individual sperm is easily discernible under all powers of the microscope. Its essential ingredients include a lipid fraction, carbohydrate, buffer salts and a gum to give the proper physical consistency. As a routine measure it is suggested that sulfathalidine, sulfasuxidine or streptomycin be added to this pabulum at the rate of 25–30 mg. per 100 ml.

In this formula glucose can be used as the only sugar but frequently the results are improved by the addition of traces of galactose. Lipositol made after the method of Woolley (14) or highly purified soybean lecithin

Lipid-glucose-buffer-gum (LGB)

Glucose	0.6	per cent
Galactose	0.2	"
KH_2PO_4	0.2	"
$Na_2HPO_4 12H_2O$	2.0	"
Lipositol, or purified lecithin	1–2.0	"
Gum acacia	3.0	"
Distilled H_2O to volume		
Sulfathalidine, sulfasuxidine or streptomycin	0.03	"

can be used as the source of the lipid. Such preparation should be freshly prepared or stored under conditions to prevent deterioration. Any of the forementioned gums may be used as desired although commercial gum acacia seemed to be one of the most desirable ones. An initial pH of 6.9 with this mixture seemed to be near optimum.

Field tests with similarly compounded dilutors, that is bases which contained glucose-galactose, lipid, gum acacia and buffer gave results comparable to yolk-buffered semen. The field results with semen largely under 36 hours in storage and under actual breeding conditions in October, 1945, gave the following:[2]

Yolk buffer semen—140 cows bred—62 fertile inseminations
L.G.B. —138 " " —65 " "

LGB can be mixed by weighing out the glucose buffer salts, lipid and gum and adding the approximate amount of distilled H_2O. Boil gently and add the antibiotic if desired. Considerable latitude in the method of making up this formula is allowable. The molarity of the solution with respect to the buffer and sugar proportions seems to lie within very narrow limits and little latitude is allowable here.

SUMMARY

The data presented suggest the following pertinent facts in relation to the storage of bull semen.

The addition of blood-fibrin, or arginine, were without measureable effect upon motility of sperm. Glucose additions in concentrations of 0.02 to 0.04 M improved in most cases the storage of bull semen environed in egg yolk-buffer. Freshly purified lecithin, or lipositol from crude soybean lecithin free of free fats or fatty acids with 0.04 M glucose, or with a mixture of 0.02 M galactose and 0.04 M glucose provided a highly effective pabulum for the storage of the sperm. In many experiments a combination of galactose and glucose (conc. indicated above) was superior to glucose alone. Inositol in 0.05 to 0.1 M solutions and in combination with glucose and galactose likewise benefited sperm motility. The addition of certain gums was beneficial while gum damar and gum guaiac were definitely toxic.

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[2] We are indebted to Dr. L. E. Casida and Mr. Geo. R. Barrett for making pregnancy diagnoses on these cows.

Certain antibiotics as well as certain of the sulfonamides were effective in controlling bacterial growth without impairment of sperm survival.

On the basis of these data a synthetic pabulum for bull sperm storage may be used. Its essential ingredients should include freshly purified lipids, specific sugars—glucose and galactose, a buffer system, a gum to supply the proper physical consistency and an agent to control bacterial contamination.

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TIME REQUIRED FOR MAKING FLAVOR JUDGMENTS OF MILK[1]

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Flavor is the major item generally evaluated in the judging of dairy products. In arriving at a flavor evaluation the judge first smells and tastes the sample and then compares the sensations noted with mental concepts of desired standards. This evaluation of flavor naturally takes some time. However, the actual time required by the experienced judge to ascertain the taste of a sample is surprisingly short. Such a judge has come to realize that the taste-reaction time not only varies with different "tastes" but also that it is fixed within certain ranges for specific tastes. Sometimes the experienced judge finds it necessary to retaste a sample, but often judgment is passed on the basis of the one taste-reaction.

Unfortunately, inexperienced judges are not appreciative of the "quickness" of taste reactions or of the sequence of sensory reactions specific to certain flavors. Consequently, the inexperienced judge may not be "in readiness," and, therefore, may miss entirely, or at least ignore, the first taste reaction. As a result, he may taste a sample repeatedly, making little progress toward final judgment. Thus, he tends to waste the precious first moments of tasting. Meanwhile, the onset of taste adaptation may make it more difficult for the beginner to diagnose correctly the often delicate, first taste sensation of the product. As a result he may tend to lose confidence in his judging ability. If he has 10 or more samples to judge at one time, he may be inclined to think that this inability to sense the taste reaction is an indication of satiation or of dulled taste sensitivity. Actually, the experienced judge, knowing when to expect taste reactions, will taste scores of samples before fatigue is experienced.

While many data have been reported on the taste-reaction time of substances in aqueous solutions yielding the fundamental tastes of sweet, sour, salt, and bitter, no such data on the flavors of milk were known to be available. Such information would appear to be of value as an aid in developing alertness in beginners in the judging of milk and for instilling greater confidence in the experienced judge. To this end data were secured in this study.

PROCEDURE

In the routine scoring of milk for flavor the opportunity presented itself for a study of the time required to place judgment on the samples. By means of a stop watch in the hands of an observer, the elapsed time from

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the introduction of the sample into the mouth until the taster signalled recognition of the flavor, was noted. While this method did not yield split-second accuracy, it did give a very good indication of the elapsed time from exposure of the sapid substance to recognition of the taste reaction, which was the desired aim.

In the majority of cases approximately 125 samples were judged at one sitting; in some instances, over 300 samples were tasted at one tasting period.

The milk consisted both of individual cow and mixed herd samples. Each sample was divided into three portions, one of which was untreated, one holder pasteurized without copper contamination, and one similarly pasteurized, but had added 0.25 ppm. of copper after pasteurization. The samples were judged at 60° F. after storing 3 and 7 days at 40° F. The identity of the samples was not known to the judge at the time of judging. All recordings were made by a second party.

Generally, only one tasting was necessary to arrive at a judgment, but in several instances when the taste reaction was not pronounced, a second or third taste was made.

The mouth was not rinsed out between samples except when an especially pronounced nauseating flavor was experienced; then tepid water was used as a rinse.

Tasting was done continuously from the start with only occasional minor interruptions. There were no rest periods following tasting of each sample or after tasting a group of samples.

RESULTS

Time recordings were taken on the judging of 2262 samples of milk for flavor. Over one-half of them rated an excellent flavor with no criticism. Interspersed were samples having "cheesy," "flat," "high-acid," "oxidized," "rancid" and "salty" flavors, the percentage distribution of which is shown in table 1.

Definite differences were noted in the average time required to recognize the various flavors in milk (table 2). For example, of the flavors observed, the judgment time of "salty" milk was found to be the least of all, being 2.9 seconds. On the other hand, more time was required to pass judgment on excellent flavor milk than on milk having various off-flavors. The order of the flavor, so far as time required to pass judgment on them is concerned, was as follows: "salty," "cheesy," "high-acid," "rancid," "flat," oxidized," "excellent."

The comparative long time required to pass judgment on excellent flavor milk may be explained by the very nature of the flavor. When off-flavors are present the taste-reaction may be noted quickly whereas in the absence of an off-flavor there is the waiting period for a possible off-flavor taste reaction and probable retasting to check the taste reaction. These average

TABLE 1

The distribution of flavors noted in 2262 samples of milk tasted

Flavor	Distribution of milk samples having specific flavors			
	Number		Per cent	
Excellent		1222		54.0
Cheesy		10		0.4
Slightly	4		0.1	
Distinctly	6		0.3	
Flat		73		3.2
High-acid		21		0.9
Slightly	13		0.6	
Distinctly	8		0.3	
Oxidized		615		27.2
Questionably (?)	61		2.7	
Slightly (+)	139		6.1	
Distinctly (++)	246		10.9	
Pronouncedly (+++)	169		7.5	
Rancid		146		6.5
Slightly	48		2.1	
Distinctly	61		2.7	
Pronouncedly	37		1.6	
Salty		175		7.7
Slightly	47		2.1	
Distinctly	128		5.6	
Total		2262		99.9

judgment times (table 2) seem to furnish proof that if the off-flavor is not sensed quickly the chances are that the milk has good to excellent flavor. It should be pointed out that the time reported in table 2 is not taste-reaction time, but flavor judgment time, which includes both the time required to get the taste reaction and the time to identify the sensation of taste. Interestingly, the times found to place judgment on milk having off-flavors embodying the fundamental tastes were in the same order of the reaction times reported in the literature (1, 2), namely, salt, sweet, sour and bitter. The off-flavors in milk having these fundamental tastes were "salty," "high-

TABLE 2

The average time required to "sense" the various flavors in milk

Flavor	Number of samples having specific flavor	Average elapsed time from tasting to passing judgment on the taste reaction (seconds)
Excellent	1222	8.2
Oxidized	615	7.0
Flat	73	6.9
Rancid	146	5.3
High-acid	21	5.2
Cheesy	10	4.9
Salty	175	2.9
Total	2262	7.2

TABLE 3

The weighted-average time to "sense" the various intensities of the oxidized flavor in milk

Intensity of oxidized flavor	Number of samples	Average elapsed time from tasting to passing judgment on the taste reaction (seconds)
Questionably (?)	61	10.1
Slightly (+)	139	9.1
Distinctly (++)	246	7.3
Pronouncedly (+++)	169	3.8
Total	615	7.0

acid" and sometimes "rancid" (bitter and rancid are not always associated), requiring judgment times in that order. Another factor which shortens the judgment time for "salty" milk is the readiness by which saltiness is identified.

The intensity of the flavor has much to do with the time of judgment as any milk judge well knows. Judgment on a pronounced off-flavor often will be made without hesitation whereas considerable time may be required to pass judgment on the same flavor of slight intensity. Data in table 3 on judgment times of milk having various intensities of oxidized flavors bear this out. In fact, the average time to judge milk finally classified as "questionably oxidized" was found to be longer than that required to judge milk classed as "excellent."

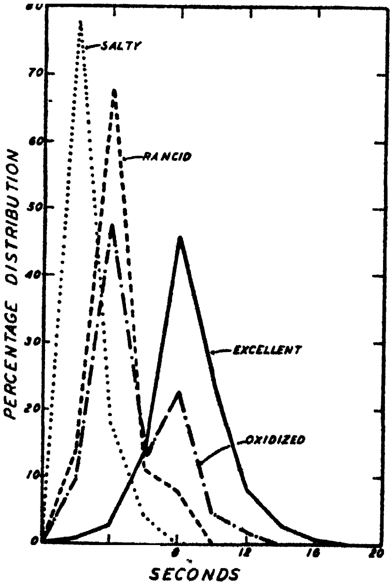

FIG. 1. The percentage distribution of the time required per sample to judge 128 samples of salty, 37 samples of rancid, and 169 samples of oxidized milk having pronouncedly intense off-flavors, and to judge 1222 samples of excellent milk.

In routine judging of milk, some factors seem to obtain which cause a considerable range in the judgment time of the sample. These may include the effects of the preceding sample, time of day, length of tasting period, rapidity of tasting and so on. Although data are not presented for all the off-flavors noted, evidence seems to show (table 2 and fig. 1) that as the average time of judgment increases the range increases also. For example, the range in judgment time for "salty" milk was from 2 to 6 seconds whereas that for "excellent" milk ranged from 2 to 16 seconds.

Percentage distribution studies showed that 78 per cent of 128 distinctly "salty" samples were judged as such in 2 seconds per sample; that 68 per cent of 37 pronounced "rancid" samples were so judged in 4 seconds per sample; that 47 per cent of 169 pronouncedly "oxidized" samples were so judged in 4 seconds per sample and that 46 per cent of 1222 samples of "excellent" milk were classed as such in 8 seconds per sample (fig. 1).

In a study of this kind, the reliability of the judging must necessarily be accepted if the data or their analysis has any merit. That the judgments are not infallible is fully appreciated. However, unpublished data on flavor studies made monthly throughout the year on many samples of milk as well as a study of the reliability of flavor judgments (1) lead one to believe that the flavor judgments reported herein may be accepted with a very high degree of accuracy.

SUMMARY

The average time required to make flavor judgment on 2262 individual samples of milk was noted. This time ranged from a low of 2.9 seconds for "salty" milk to 8.2 seconds for "excellent" milk. The judgment times for "cheesy," "high-acid," "rancid," "flat" and "oxidized" milk were between these extremes and were in the above order. The average judgment time for the 2262 samples was 7.2 sconds per sample. Milk having off-flavors of slight intensity required considerably longer judgment time than did those having pronouncedly intense off-flavors. "Excellent" flavor milk invariably required longer judgment time than did off-flavor milk.

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HEAT COAGULATION OF MILK

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The need for further studies on heat coagulation became apparent during attempts to measure the influence of various electrolytes upon the coagulation temperature of various milks. The data available proved rather limited, and attempts to check certain published results were only partially successful.

A lack of uniform methods, it is felt, largely accounts for the differences obtained by various workers. In the present study, the method adopted was carefully standardized, in an attempt to minimize the error in judgment regarding the point at which coagulation first occurs. Goat's milk as well as cow's milk was used.

REVIEW OF LITERATURE

Hammersten (2) reported that the coagulation temperature of milk varied between 130° and 150° C. Benton (1) found wide variations in the coagulation characteristics of milk from different quarters of the same udder. Sommer and Hart (6), who studied the heat coagulation of milk from individual cows, at 136° C., found that the time of coagulation ranged from 90 seconds to 20 minutes. In their opinion, the main reason for these differences was the relative amounts of calcium and magnesium as compared with citrates and phosphates in milk. Their findings have since been confirmed by various workers.

Rogers, Deysher, and Evans (5) found no correlation between the coagulation temperature of raw mixed herd milk and that of the same milk after evaporation. Subsequent work has substantiated this claim. Holm, Deysher, and Evans (3) determined the relation between the coagulation temperature and the coagulation time (which was 10 to 60 minutes). They found approximately a logarithmic relationship with respect to time. Further, they pointed out that unless calculations were made upon the concentration of milk-solids-not-fat in the fat free portion of the sample, deviations from this relationship might be expected. Later, Webb and Holm (8) studied the effect of various temperatures (80° to 120° C.) upon the coagulation time of several milks containing 9 per cent and 18 per cent milk-solids-not-fat. According to results, as the temperature is increased the coagulation time decreases logarithmically, though the rate of decrease is greater for milks with 18 per cent milk-solids-not-fat than for those with 9 per cent.

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Miller and Sommer (4), studying the coagulation in sealed glass tubes, found that milks were very sensitive to pH changes between pH 6.4 and 6.2 and, to a lesser extent, between pH 5.4 and 5.2. According to them, the coagulation curve was displaced by the presence of added salts as well as by preheating or concentrating. Webb, Bell, Deysher, and Holm (7) found that the difference in stability caused by acid or salts was one of degree; it affected the general heat-stability level rather than the stability interrelationships of milks forewarmed one way or another.

EXPERIMENTAL

Determinations were made for coagulation time of milk samples sealed in glass tubes and heated at various temperatures over the approximate range of 110° to 160° C. Each sample occupied about 40 per cent of the volume of the sealed tube, which was 8 mm. in diameter and 11 to 12 cm. in length. By rocking these tubes slowly during heating in an oil bath, one could easily ascertain the time required for coagulation to occur. Two thermostatically controlled baths were used in the study. They could be regulated to operate at any desired temperature within the range indicated, with a maximum variation of ± 0.05° C. The tubes containing the samples were mounted on a rocker with clips for 8 tubes. By connecting the arm of the rocker to a motor-driven rotor, one could obtain the desired flow in the tubes. The device was adjusted so that the samples flowed from one end to the other every 20 to 25 seconds, and the tilt was so regulated that agitation was not extreme. Coagulation was judged by observing the point at which the curd particles or clots appeared in the tubes. The procedure was carefully adjusted and maintained throughout the experiments, since, during standardization, the rate of rocking as well as the extent of tilt was found to influence the time of coagulation at a given temperature.

The procedure used somewhat resembles that employed by Miller and Sommer (1940), except that in this study no attempt was made to determine the temperature at which coagulation occurred in a stipulated time. Instead, the time required for coagulation at a given temperature was observed.

Duplicate bath assembles were employed, since preliminary observations showed that satisfactory duplicate results could be obtained with the two units. By heating the same samples simultaneously in two baths at different temperatures, one could obtain the desired data on a given set of samples within 2 days. Properly refrigerating the samples during this period, except while they were being heated, minimized the likelihood of significant changes other than those produced by the heating itself.

The samples used for coagulation studies were analyzed for fat content by the Babcock test, for total solids content by the Mojonnier method, for pH by the Coleman pH meter, and for titratable acidity by titrating 9 ml. of milk plus 9 ml. of water with about N/10 NaOH, and calculating the results as lactic acid.

Samples of milk from individual cows were obtained from the University dairy herd; those of herd milks, from various dairies and from patrons delivering to a market-milk plant. The latter were selected to give predominantly milk from certain breeds or milk from several breeds, according to factors under consideration. A few samples of goat's milk were also included. These were obtained from a dairy milking about 100 goats with three breeds represented.

Considerable variation occurred in the coagulation time of various samples at a given temperature. This finding agrees with results published earlier. Thus at 115° C. the milk from cow 562 coagulated in 151 minutes, whereas that from cow 1076 required 480 minutes. With a set of samples, however, the coagulation time at one temperature was not necessarily an index to the order of coagulation at a different temperature. Sometimes, for instance, with 8 samples at approximately 110° C., the sample that required the longest time to coagulate would be the first to coagulate at about 160° C.; or, vice versa, the first to coagulate at the lower temperature may be the last to coagulate at the higher. Considerable variation, therefore, exists in the nature of the coagulation curves for different samples of milk; and the relative stabilities of samples at one temperature do not indicate what their relative stabilities will be at another.

Table 1 gives coagulation data on one set of samples from individual cows and illustrates the variation just noted. Somewhat similar data for mixed herd milks were obtained; but the results indicate a greater likelihood of obtaining extreme variations with milks from individual cows than from mixed herd milks.

The determinations for fat, total solids, titrable acidity, and pH are given in table 2 for the samples tabulated in table 1.

Unfortunately, coagulation data are not available for individual cows throughout lactation. Data are available, however, for three cows for January, March, and April. Figure 1 clearly indicates that the milk from cow 787 was more heat stable in March and April than in January. With the

TABLE 1

Influence of temperature on coagulation time

Sample No.	Cow No.	Coagulation time at indicated temperatures						
		108.6° C.	115.0° C.	121.5° C.	130.3° C.	140.3° C.	150.7° C.	160.0° C.
		min.	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
1	562	222	151	85	43.0	17.4	7.2	2.33
2	787	220	192	120	60.0	26.8	11.7	3.17
3	800	284	153	88	40.0	23.3	6.5	2.75
4	805	253	153	83	50.3	22.9	9.3	2.83
5	838	324	191	121	54.7	23.8	9.3	2.25
6	1074	275	192	94	51.1	11.8	4.6	2.00
7	1075	380	206	122	49.8	20.0	7.2	2.33
8	1076	720	480	244	107.0	35.7	3.2	1.50

TABLE 2

Composition, titratable acidity, and pH values on samples used in table 1

Sample No.	Cow No.	Fat	T.S.	Titratable acidity	pH	
		%	%	%	*4–3–45*	*4–5–45*
1	562	4.8	14.68	0.21	6.50	6.48
2	787	5.0	16.65	0.21	6.60	6.60
3	800	5.8	15.75	0.23	6.45	6.42
4	805	6.9	17.50	0.24	6.55	6.54
5	838	4.6	14.15	0.23	6.50	6.50
6	1074	4.0	12.77	0.20	6.55	6.53
7	1075	3.8	12.20	0.19	6.50	6.45
8	1076	3.5	11.90	0.17	6.60	6.57

other two animals, the differences are less great; in fact, their milks are slightly less stable in April than in January, and most stable in March. Differences in stage of lactation or, possibly, differences in feeding may account for part of these variations; but individual cow differences undoubtedly also play a part. This latter point is illustrated in figure 2, giving data for cows 838 and 1076.

Figure 3 shows results with one set of mixed herd samples. If the logarithm of the coagulation time for these samples is plotted against temperature, significant variations from a straight-line relationship occur. Such a plot has been made in figure 4 for one set of samples from individual cows. The data from three such series (7 or 8 samples per series) were examined along with one series of mixed herd milk to see whether they could be made to fit a straight line when the logarithm of coagulation time is plotted against coagulation temperature.

For each curve, representing the experimental data for one milk sample, a straight line was drawn through two points established by averaging the experimental values for each half of the curve. Since the curves did not all

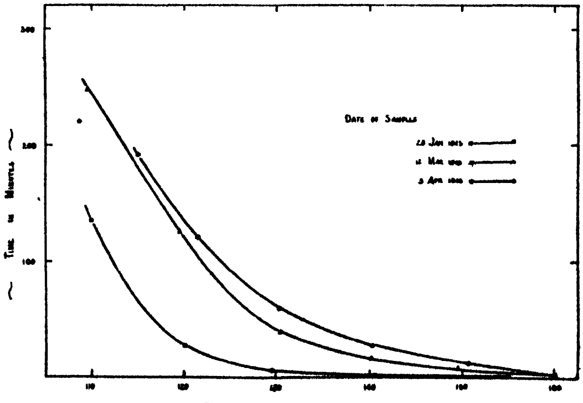

FIG. 1. Variations in heat stability of milk from cow 787.

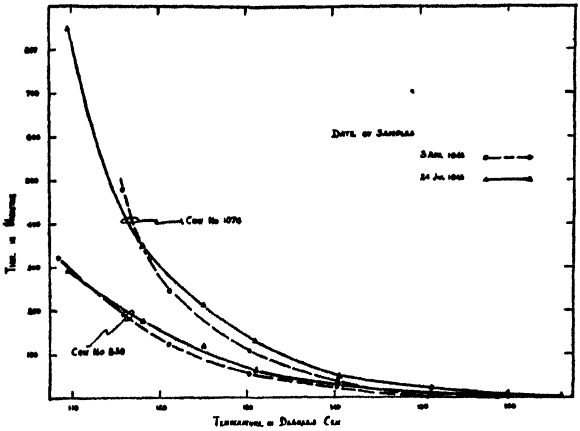

FIG. 2. Differences in heat stability of milk from cows 838 and 1076.

include the same number of established points, the averages used were data from three to five experimental points, for that part of the curve under consideration. The center part of the curve was weighted somewhat by overlapping one or two points in arriving at the averages noted. For each sample, a curve was drawn showing the deviations from this straight line. These differences were then averaged for each series of samples, and figure 5 gives the deviations. The curve for April 2 in figure 5 represents the set of samples shown in figure 4.

In all the series, the variations are consistent. Even if the straight lines considered were drawn by inspection, the deviations would give curves of the type indicated. In other words, the line that best fits the data deviates significantly from a straight line. Although not presented, the fourth series shows the same type of curve.

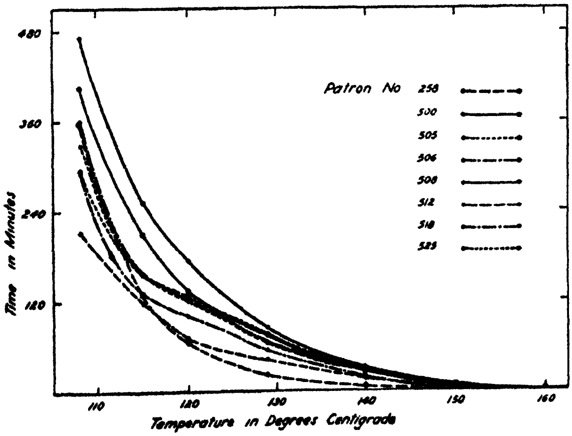

FIG. 3. Relation of coagulation time to coagulation temperature for 8 samples of of mixed herd milk.

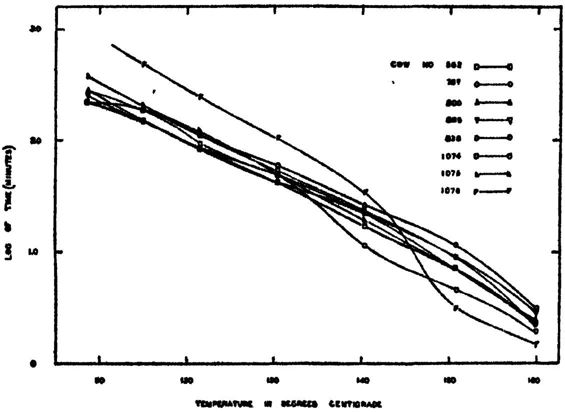

FIG. 4. Relation of logarithm of coagulation time to coagulation temperature for 1 set of samples from individual cows.

Goat's milk shows a greater deviation in this regard than cow's milk. When data for such milk are plotted, as in figure 6, with logarithm of coagulation time and coagulation temperature as coordinates, then deviations from a straight line are so apparent that no further plotting seems necessary. Most of the samples, however, show a fairly straight-line relationship if only parts of the curves are selected for consideration.

In comparing the data in table 4 with those in table 1, one sees that the coagulation times for goat's milk at 134° C. are about the same as they are for cow's milk at 155 to 160° C. At about 110° C., on the other hand, goat's milk coagulates no faster than cow's milk.

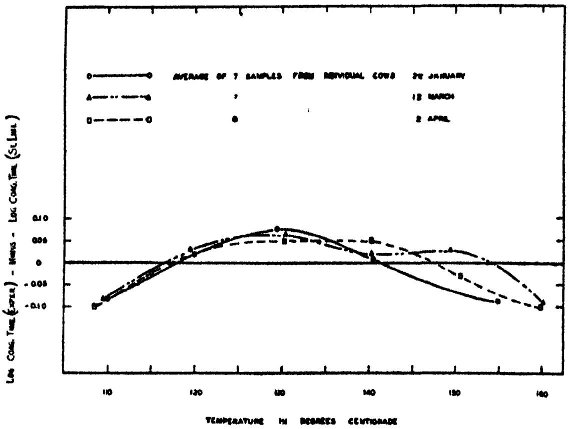

FIG. 5. Deviations from a straight line of "log time-temperature" relationship for 3 sets of samples from individual cows.

TABLE 3

Data on goat milk samples used for coagulation tests

Sample No.	Goat name	Bacteria per ml.	Titratable acidity	pH	Fat	Total solids	M–S–N–F
			%		%	%	%
1	Composite—10-gal. can	5150	0.13	6.54	3.7	11.88	8.18
2	Geraldine	325	0.15	6.54	4.4	13.15	8.75
3	Sophie	250	0.13	6.60	4.2	12.52	8.32
4	Zella	105	0.18	6.31*	3.0	11.09	8.09
5	Victoraune	1190	0.12	6.60	3.8	12.48	8.68
6	Eureka	150	0.11	6.69	3.9	11.68	7.78
7	Rosebud†	695	0.12	6.67	3.4	11.44	8.04
8	Paloma	90	0.12	6.61	3.2	11.10	7.90

* No explanation is available to account for the low pH of this sample.

† Rosebud freshened June 5, 1943; and she was milked twice daily from then until this sample was collected on July 2, 1945.

DISCUSSION

The complexity of the phenomenon of heat coagulation of milk is re-emphasized by the data presented. Lack of agreement among various workers is probably due—in part, at least—to differences in methods of determining coagulation. All agree, however, that there are marked variations in the heat stability of different milks.

Because of variations in the characteristics of the curd or coagulum formed when milk is heated, it is sometimes difficult to ascertain just when coagulation occurs. This is true when a very soft curd is produced, since only slight agitation may be required to break up the curd. If, on the other hand, a firm curd is produced, the coagulation point is easily ascertained. In this report differences in the type of clot or coagulum are not noted, although they were regularly observed throughout this study. The character of some clots may be more important than the time required to produce them. Workers should recognize that marked differences do occur in this regard.

TABLE 4

Influence of temperature on coagulation time for goat milk

Sample No.	Goat name	Time of coagulation at indicated bath temperature					
		109.5° C.	115.2° C.	120.5° C.	127.3° C.	134.0° C.	140.5° C.
		min.	*min.*	*min.*	*min.*	*min.*	*min.*
1	Composite—10-gal. can	525	335	202	94	1.5	0.6
2	Geraldine	424	300	188	113	2.0	0.4
3	Sophie	558	363	249	140	3.5	0.6
4	Zella	848	450	61	5.5	0.5	0.5
5	Victoranne	548	346	249	130	4.5	0.9
6	Eureka	466	339	227	138	1.0	0.8
7	Rosebud	537	349	231	130	7.5	1.8
8	Paloma	820	460	32	5	1.2	0.4

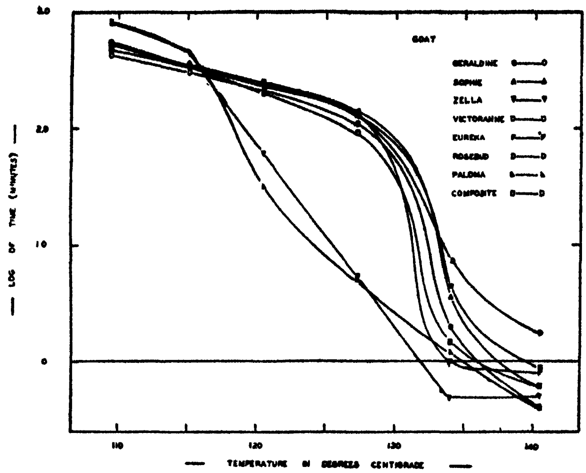

FIG. 6. Relation of logarithm of coagulation time to coagulation temperature for 8 samples of goat's milk.

Variations in heat stability are shown for milk from individual cows and mixed herd milk at the same season of the year, as well as differences in heat stability of milk from the same cows at different seasons. The data also show that for a series of samples tested simultaneously, the relative stabilities to heat are apt to vary according to the temperatures of observation. That is, the order of coagulation may be different at one temperature than at another.

According to Holm, Deysher, and Evans (3), when the concentration of milk-solids-not-fat in the fat-free portion is considered, there is a logarithmic relationship between coagulation time and coagulation temperature. In the present experiments, a consideration of concentration of milk-solids-not-fat in milk as it is normally secreted did not change the relationship already shown—namely, that there is a significant deviation from a straight line when a sufficient temperature range is considered.

There are interesting differences in the heat coagulation characteristics of milk from cows and goats. What constituents are responsible is not clear; but, since slight changes in certain components of milk may cause significant changes in heat stability, these differences are in keeping with expectations.

SUMMARY AND CONCLUSIONS

1. A method of determining coagulation times at various temperatures is described, and results obtained over the approximate temperature range of 110° to 160° C. are reported for both cow's and goat's milk.

2. Marked differences occurred in the nature of the coagulum produced as a result of heating various samples of milk.

3. Considerable variation was also found between the samples of cow's milk so far as coagulation time at a given temperature was concerned. Somewhat similar differences were observed between samples of goat's milk.

4. There seemed to be a characteristic difference between cow's and goat's milk, since coagulation times for goat's milk at 135° C. are about the same as for cow's milk at 155° to 160° C. Other differences are also pointed out.

5. Although, over a limited temperature range, most samples show a fairly straight-line relationship between coagulation temperature and logarithm of coagulation time, there was considerable variation from this relationship when the approximated temperature range of 110° to 160° C. was considered.

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MERCURIMETRIC METHOD FOR SALT DETERMINATION IN BUTTER AND NEW CHEESE

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Accurate determinations of the salt content of dairy products are important because of the effect which salt has upon the flavor and quality of these products. The chlorine content of fresh milk is often used as a basis for judging the quality and detecting physiological abnormalities of milk at the intake in market milk plants. The Volhard and Kohman methods are generally used for making chloride determinations of dairy products. Several investigators have recommended making modifications of these methods on the basis that it is difficult to detect the point of neutralization in the titration technique used. This allows considerable latitude for interpretation of results and it is not unusual for the results obtained from different laboratories to vary widely.

REVIEW OF LITERATURE

A complete review of the literature concerning the determination of chlorine in milk is given by Sanders (5). Sanders (5) proposed a modification of the Volhard method for determining chlorine in milk and describes the sources of error and methods of improvement in other methods, as well as conditions necessary for accurate Volhard titrations.

Skelton and Bryant (7) suggested the use of dichlorofluorescein as an indicator, in order to secure a more distinct and sudden end point in the titration procedure in either the official or factory method for determination of salt in butter.

Marquardt (3) has pointed out the need for a simple usable procedure for rapidly determining the salt percentage in freshly made commercial cheeses of all varieties and suggested a procedure based upon the Sammis method (4). He shows that it is possible to obtain reliable results by titrating directly aliquots of solutions prepared from freshly made cheeses less than five days old. The results secured on several varieties of fresh cheese compared favorably with those obtained by the modified Volhard method (8), approved by the American Dairy Science Association. He also showed that potassium chromate and dichlorofluorescein gave comparable results when used as indicators for the direct titrations with silver nitrate, but it was not possible to obtain accurate values with cured cheese by direct titration because water-soluble proteins rendered this procedure unsuitable for certain cheese over five days old.

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A mercurimetric titration method, using s-diphenylcarbazone as an indicator, has been suggested by Schales and Schales (6) for determining chlorine in biological fluids. It is stated that this method has been found to be free from errors that are frequently encountered in other chloride determination procedures. The chloride combines with mercuric ions, according to the equation, 2 Cl′–Hg″, without forming a precipitate. Mercuric chloride is very slightly disassociated, and the end point of the titration is therefore easily recognized by the appearance of mercuric ions in solution.

The purpose of this investigation was to establish the value of a mercurimetric method, using the s-diphenylcarbazone indicator as a test for chlorides and salt in milk and milk products.

PROCEDURE.

A study was made to determine if the mercurimetric method, using s-diphenylcarbazone as an indicator, could be modified for use as a laboratory or plant method in determining the chloride content of fresh milk. Special attention was given to the various factors, such as concentration of solutions, amount and method of treating sample and amount of indicator, upon the results secured.

A further study was made to determine if the mercurimetric method could be modified for use as a laboratory or plant method in determining the salt content of butter, American cheese and soft cheese. A number of commercial samples of the various products were analyzed for salt content by the mercurimetric method. The results secured were compared with those secured by commonly used methods and the accuracy of the method was checked by recovery tests on samples of the product to which a known amount of salt had been added.

Lastly, an attempt was made to establish the value of the mercurimetric method for miscellaneous uses in the dairy industry.

RESULTS

No satisfactory method was found for using the mercurimetric method in determining the chloride content of fresh milk. Various methods of preparing the sample were employed including making different dilutions of the sample, using different amounts of the sample and also ashing of the sample. Varying amounts of indicator ranging from 0.1 ml. to 1 ml. for a 10-ml. sample were used and solutions of different concentration were used but a definite color change at the neutralization point was not secured.

The procedures which gave satisfactory results for estimating the salt content of butter, new cheese and cottage cheese by the mercurimetric method are as follows.

Reagents and Procedures

Mercuric nitrate solution—0.1711 N. Dissolve 29.31 g. of mercuric nitrate C.P. in a few hundred ml. of water with the addition of 40 ml. of 2 N nitric acid and make the solution to one liter volume with distilled water. The correct amount of nitric acid must be used in preparing the standard mercuric nitrate solution since if more or less acid is used the end point in the titration technic will not be as sharp.

Nitric acid—2 N. Dilute approximately 127 ml. of concentrated nitric acid (about 16 N) to one liter volume with distilled water. Use to prepare the mercuric nitrate solution.

s-Diphenylcarbazone indicator solution. Dissolve 100 mg. of s-diphenylcarbazone (Eastman 4459) in 100 ml. of neutral ethyl alcohol and store in a dark place preferably in a refrigerator. The correct amount of indicator must be used and best results are secured if the indicator is stored not longer than one month.

Standard sodium chloride—0.1 N. Dissolve 5.845 g. of sodium chloride C.P., dried at 120° C., in water and make to one liter volume. Use the standard sodium chloride to standardize each new solution of mercuric nitrate. Standardize each new solution of mercuric nitrate by measuring 25 ml. of the standard sodium chloride solution into a 125-ml. Erlenmeyer flask. Add 0.6 ml. of indicator solution and titrate slowly with the mercuric nitrate solution. The clear solution turns a pale violet at the end point and an intense violet-blue color on the addition of one drop of mercuric nitrate in excess.

In protein-free solutions the color change is as described above. In protein-containing solutions the color of the turbid liquid is salmon red after the addition of the indicator, but changes as the titration is started, to violet. As the titration proceeds the solution becomes clear and pale yellow to colorless and at the end point there is a sharp change to pale violet.

Estimation of the Salt Content of Butter

Weigh a 10-g. sample of butter into a counterpoised beaker. Secure 250 ml. of washings from the sample as directed in the Official Method (2) for determining salt in butter. Or for plant laboratory procedure, after the moisture and fat determinations have been made by the Kohman method, rinse the dry residue into a 250-ml. volumetric flask with three separate 30-ml. portions of hot distilled water. Cool the rinsings; dilute to the 250-ml. graduation mark on the flask and mix thoroughly.

Pipette a 25-ml. portion of the solution into a 125-ml. Erlenmeyer flask. Add 0.6 ml. of *s*-diphenylcarbazone indicator. Titrate slowly with standard mercuric nitrate solution using a burette graduated in 0.1-ml. divisions. Single drops should be approximately 0.05 milliliter. The clear and colorless solution turns a pale violet color on the addition of one drop of mercuric nitrate at the end point and an intense violet-blue color on the addition of one drop of mercuric nitrate in excess. The ml. of 0.1711 N mercuric nitrate used in the titration equal the percentage salt in the sample.

Estimation of the Salt Content of New Cheese and Cottage Cheese

Grind the sample of new cheese or cottage cheese finely to reduce to a homogeneous consistency. Weigh 10 grams of the prepared sample into a 400-ml. beaker. Add 250 ml. of distilled water and heat to 150–160° F. Mix thoroughly and allow to cool to room temperature. Remove a 25-ml. portion of the clear solution and place in a 125-ml. Erlenmeyer flask. Titrate with mercuric nitrate as described for butter. The ml. of 0.1711 N mercuric nitrate used equal the percentage of salt in the sample.

Recovery Tests

Tests were made using the procedure suggested above to determine the correct color change to use as the end point in the mercurimetric method. Samples of butter of exactly known salt contents were examined by the mercurimetric method. The readings were taken upon the occurrence of the pale violet color just before one more drop of mercuric nitrate produced the intense violet-blue color. The reading was also taken upon the occurrence of the intense violet-blue color which represented one drop of mercuric nitrate in excess. Table 1 shows the results obtained.

TABLE 1

The relation of color change in the mercurimetric procedure to the exact salt content of butter

Sample	Known amount of NaCl	Mercurimetric method	
		Pale violet color NaCl	Intense violet-blue color NaCl
	percentage	*percentage*	*percentage*
1	0.994	0.99	1.03
2	1.987	1.98	2.02
3	2.981	2.95	3.00
4	3.974	3.98	4.04
5	5.026	5.00	5.05
Ave. variation from exact amount		– 0.015	+ 0.036

The results given in table 1 show that the most accurate results are secured by taking the reading upon the occurrence of the pale violet color just before an extra drop produced an intense violet-blue color. The pronounced violet-blue color may be used as an end point with only slightly higher results.

Table 2 shows the agreement obtained in comparing the mercurimetric method and the silver nitrate-potassium chromate method in recovery tests on samples of butter and cheese containing known added amounts of salt. The silver nitrate-potassium chromate method for the butter examination was the modified Kohman method approved by the American Butter Institute (1), and the method used for the determination of the salt content of cheese was that suggested by Marquardt (3).

Table 2 shows that the results secured on recovery tests by the mercurimetric method compared favorably with those secured by the silver nitrate–potassium chromate method.

The salt content of a number of samples of butter and new cheese was determined by the mercurimetric and the silver nitrate–potassium chromate methods. The butters varied in quality and ranged in salt content from 1.5 to 3.0 per cent. The cheese varied from 0.5 to 4.5 per cent in salt content and were composed mostly of new cheddar and cottage cheese, although a few other varieties were included.

TABLE 2

Recovery tests on butter and cheese of known salt content by the mercurimetric and the silver nitrate-potassium chromate methods

Sample	Known added amount of NaCl	Mercurimetric method NaCl	Silver nitrate-potassium chromate method NaCl
	percentage	*percentage*	*percentage*
Butter			
1	0.994	0.99	1.02
2	1.987	1.98	2.04
3	2.981	2.95	3.06
4	3.974	3.98	3.99
5	5.026	5.00	5.08
Cheese			
1	0.986	0.97	1.00
2	1.972	1.97	2.00
3	3.016	3.00	3.05
4	4.002	4.01	4.02
5	4.988	4.95	5.02

The results secured by the two methods are shown in table 3 and indicate that the mercurimetric method gives somewhat lower results than the silver nitrate–potassium chromate method.

Observations indicated that in the cheese samples examined, the water-soluble protein was less likely to cause inaccurate results when the mercurimetric method was used. Lower and more accurate results were secured for both the butter and the cheese samples when the mercurimetric method was used. In cheese where considerable water-soluble protein is present, the color change with the mercurimetric method is that as described for protein-containing solutions.

Miscellaneous Application

An attempt made to establish a method for the use of the mercurimetric

TABLE 3

Salt determinations of butter and cheese by the mercurimetric and the silver nitrate-potassium chromate methods

Kind of sample	Number of samples	Mercurimetric method NaCl	Silver nitrate-potassium chromate method NaCl
		Ave. percentage	*Ave. percentage*
Butter	25	2.77	2.81
Cheese	17	1.34	1.44

method in determining the chlorine content of commercial preparations and chlorine rinsing solutions failed to produce a satisfactory procedure.

A procedure was found which gave satisfactory results for use in detecting the presence of mercuric chloride in preserved samples of milk. The sample of milk was thoroughly mixed. One ml. of the milk sample was removed for examination. Four drops of the *s*-diphenylcarbazone indicator, prepared as previously described, were added to the 1-ml. portion of the sample removed. The occurrence of a violet-blue color indicated the presence of mercuric chloride. A definite color change was produced by as little as one part of mercuric chloride in 100,000 parts of milk.

DISCUSSION AND CONCLUSIONS

The mercurimetric method for salt determination as reported in this paper is somewhat more delicate, gives accurate results and has the advantage of giving a sharp and permanent end-point in comparison to titrating with standard silver nitrate using potassium chromate as an indicator. An intense violet-blue color occurs upon the addition of one drop of mercuric nitrate in excess. Mercuric nitrate is considerably cheaper than silver nitrate, but the *s*-diphenylcarbazone is not as readily available as potassium chromate as an indicator.

The results indicate that the mercurimetric titration method can be successfully adapted for use as a salt test for butter and new cheese and the *s*-diphenylcarbazone indicator can be used in a procedure for detecting the presence of mercuric chloride in milk. The mercurimetric method does not seem to be adaptable to procedures requiring digestion of the sample, and no satisfactory procedure was found for using the mercurimetric method as a direct titration procedure for determining the chlorine content of milk, nor for determining the chlorine content of chlorine sterilizers used in the dairy industry.

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CRYOPHILIC ORGANISMS IN WATER AND BUTTER

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INTRODUCTION

Any organism capable of growth at refrigerator temperatures may be important in the keeping quality of dairy products. It is recognized that many organisms, which exhibit optimum growth at room temperatures or above, may grow in the vicinity of 5° to 10° C., but the most important are those true cryophiles which have their optimum growth in that range.

In spite of the extensive use of low temperatures for storing dairy products, analyses in the bacteriology laboratory and keeping quality tests most frequently employ incubation temperatures of 20° to 37° C. While certain time-saving advantages are offered by the use of higher temperatures, it is well to recognize that the use of incubation temperatures more closely approaching storage conditions are more apt to give results which represent changes occurring under commercial conditions. It is admitted that from the public health standpoint, 37° C. incubation may be desirable, but for purely commercial reasons, it is stressed that the use of higher incubation temperatures may give a false picture of what happens under storage conditions.

REVIEW OF LITERATURE

The available literature reveals the fact that a number of defects of dairy products may be attributed to the action of cryophilic organisms.

Several investigators (6, 14, 22, 30, 31) have attributed ropiness in milk to organisms showing active growth in the neighborhood of 10° C. In a number of cases the organisms at fault were traced to water supplies.

Pigment production at low temperatures may take place in milk and cream on rare occasions, but commercially it is more important so far as butter is concerned. Several papers (19, 26, 43, 44) have reported organisms isolated from butter which were capable of rapid pigment production at low temperatures. *Pseudomonas nigrafaciens* was concluded to be the causative organism in some of the cases cited.

Cryophiles, both obligate and facultative, may be responsible for the failure of fluid dairy products to meet commercial and public health bacterial standards even though the products in question are handled and stored according to approved procedures (17, 27, 28, 42). Cryophilic bacteria

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may cause milk samples to show unsatisfactory methylene blue tests. High counts have been caused by the vigorous growth of organisms after the cream has been pasteurized, bottled, and stored at refrigerator temperatures. Cream shipped from Midwestern creameries to Eastern markets has been threatened with rejection because of high bacterial counts. The increase in count has taken place during shipment in refrigerated cars. A number of cryophilic organisms have shown phosphatase activity, and consequently the phosphatase test should be used with discretion on butter which has been subjected to a holding period which would allow considerable growth.

Organisms growing at low temperatures may be responsible for off-flavors and odors in all dairy products, but chiefly butter, because of the length of time it may be stored at temperatures just above freezing. The spoilage of milk and cream standing at low temperatures is characterized principally by proteolysis and the development of a bitter flavor (16, 36). Butterfat likewise may be attacked at refrigerator temperatures and cream spoilage is common (2, 3, 24).

Rancidity in butter may be caused by the action of microorganisms growing at low temperatures (13, 22, 39). Active growth in the region of 5° C. has been accompanied by the development of rancidity in artificially seeded butter.

It has been shown (18) that cheesy flavors develop quickly at low storage temperatures, and that extensive bacterial growth takes place in the vicinity of 5 to 10° C. The use of higher storage temperatures (21° C.) resulted in defects not typically cheesy and only slightly resembling those developed at the lower temperatures.

Surface taint is another defect of butter appearing at low temperatures (10, 12, 13, 15, 21). Organisms producing such defects have been traced to defective water supplies in many instances (10, 11, 15, 18, 21, 25, 32, 34, 35, 38, 41). These organisms, in a majority of cases, apparently belong to the genera *Pseudomonas* and *Achromobacter*. Several workers (20, 23, 29, 31) have stressed the importance of types, rather than numbers, present in butter since the temperature of holding influences the growth of microorganisms in the product in question.

The use of low incubation temperatures for analyses of creamery water supplies is advocated in several papers (8, 23, 45), and it has been shown that low temperature incubation gives in some cases vastly different results than incubation at high temperatures.

EXPERIMENTAL PROCEDURE

Water samples for the analyses were obtained from Minnesota and Wisconsin creameries which had been experiencing trouble with butter which kept poorly. Butter samples were obtained from a number of sources. All showed a pronounced defect which either appeared in a print of butter under commercial conditions or which resulted when various butters were subjected to a routine keeping-quality test. Only samples which developed a type of defect ostensibly the result of bacteriological action were selected.

The basal medium from which the various other media were prepared for making differential counts of lipolytic and caseolytic types of organisms was essentially the "Standard Methods" agar (1) from which the skim milk was omitted. For determining lipolytic types, nile blue sulfate-butterfat was used and prepared according to Knaysi's method as cited by Stark and Scheib (40). Caseolytic types were counted on a skim milk agar prepared by adding, at the time of plating, 5 ml. of sterile skim milk to 100 ml. of the basal medium, melted and cooled to 45° C.

Water and butter samples were plated in suitable dilutions for the determination of total, lipolytic, and caseolytic counts. Duplicate plates of each dilution were incubated at 37° C. for two days, 20° C. for five days, and 8° C. for seven days, respectively. In addition, the water samples were inoculated into brilliant green-lactose-bile broth as a presumptive test for the presence of coliform organisms, using 1.0 and 0.1 ml. inocula, and with incubation for two days at 37° C.

Well-isolated colonies showing typical lipolytic and caseolytic reactions on the plates incubated at 8° C. were picked from the plates and inoculated into litmus milk. The litmus milk tubes were allowed to incubate for five days at 8° C. The cultures were then streaked on plates of the basal medium. A discrete colony, typical of the culture on the plate, was again picked for seeding into litmus milk and the process repeated in order to get a pure culture.

Methods and media used for the identification of the cultures were mostly those suggested in the "Manual of Methods for Pure Culture Study of Bacteria" (37). Differentiations were made on the basis of morphological and biochemical characteristics. In addition to the above, the growth temperature range and tolerance, pasteurization survival, and the presence of an oxidase enzyme were also determined. Growth temperature range and tolerance were determined by inoculating slants of the "Standard Methods" agar (1) and incubating at 8°, 20°, 37°, and 50° C. Observations on the presence and relative amounts of growth were made at the end of two, five, and seven days incubation.

Pasteurization survival was determined after inoculating two-day-old pure cultures into skim milk, holding at a temperature of 65.5° C. (150° F.) in a constant temperature bath for thirty minutes, cooling, and then preparing streak plates to determine the presence of any viable organisms. Oxidase-producing organisms were detected by the use of p-aminodimethylanaline monohydrochloride, according to the method suggested by Castell and Garrard (7).

Bergey's *Manual of Determinative Bacteriology* (5) was the basis for identification.

All of the cultures identified were inoculated into sterile cream to determine the type of defects they were able to cause at 8° and 20° C. Samples of cream incubated at the above temperatures were examined at regular intervals for type and intensity of defect produced. Further work was attempted using a sample of decomposed cream, consisting of a mixture of these incubated samples. The cream was distilled under vacuum, which resulted in a boiling point of 43° to 47° C. The distillate from the decomposed cream was compared with that from a sterile control sample held under the same conditions. Qualitative tests for the presence of sulfides, mercaptans, and sulfates, as well as a nitro-prusside test, were made on the distillate.

Five pure cultures selected on the basis of the type and intensity of proteolytic defect produced were inoculated into larger amounts of cream and skim milk. The samples were judged for type and intensity of defect, and the acidity and a formol titration as proposed by Clark and associates (9) were run on each culture. The remainder of the sample was then vacuum distilled as mentioned previously. Tests were made for the volatile sulfides and mercaptans which did not condense in the distillate. The distillate was compared with the original cream for the presence of odors and was examined qualitatively as previously stated. In addition, the distillate was examined quantitatively for mercaptans by a modification of the method of Bell and Agruss (4). A Coleman glass electrode pH electrometer, model 3D, was used for pH readings and in titration procedures.

RESULTS

I. *Bacterial counts on water and butter samples.* It is a well-known fact that the temperature of incubation influences the number of colonies appearing on a plate. The data are presented to compare the effects of the three incubation temperatures used on the demonstrable flora present in the samples examined. As would be expected, the water samples showed considerable variation both in total and differential counts.

Table 1 presents the results obtained on the water samples. A number of the waters plated had counts at all temperatures which were so low as to be quite insignificant. However, a number of the waters, particularly the untreated samples, showed considerable counts. In a majority of the samples plated, 37° C. incubation gave lower counts than incubation at 20° C. In general, it is obvious that 37° C. incubation may not reveal a count representative of the sample in question. This statement is supported by the observation that in no instance did 37° C. incubation give a maximum count. Caseolytic and lipolytic counts obtained at 37° C. showed the same relationship as the total counts. Also, it is to be noted that only in a few

TABLE 1

Bacterial counts of water samples

Sample	Incubation temperature	Total count	Proteolytic count	Lipolytic count
	°*C.*	*per ml.*	*per ml.*	*per ml.*
1	37	178	61	1
	20	410	76	10
	8	61	10	10
2	37	10	1	1
	20	16	10	1
	8	10	1	1
3	37	11	10	1
	20	36	10	10
	8	13	1	1
4	37	11	10	1
	20	14	10	1
	8	10	1	1
5	37	17	10	1
	20	76	10	10
	8	10	1	1
6	37	10	1	1
	20	28	10	1
	8	x	x	x
7	37	45	14	1
	20	76	22	10
	8	x	x	x
8	37	10	1	1
	20	164	35	18
	8	x	x	x
9	37	10	1	1
	20	20	10	1
	8	x	x	x
10	37	2,180	370	37
	20	38,000	13,500	610
	8	16,700	4,000	480
11	37	62	1	1
	20	3,270	33	250
	8	3,450	37	1,120
12	37	162	11	1
	20	32,600	230	14,100
	8	TNTC	38	680
13	37	1	1	1
	20	20	1	10
	8	10	1	1
14	37	1	1	1
	20	10	1	10
	8	1	1	1
15	37	280	40	23
	20	830	110	83
	8	322	90	36
16	37	1	1	1
	20	38	10	1
	8	10	1	1

(x) Plates discarded because of SO_2 leak in incubator. TNTC—too numerous to count in 1: 100 dilution.

instances were considerable caseolytic and lipolytic counts demonstrated at 37° C.

The presumptive tests for the presence of coliform organisms using brilliant green-lactose-bile broth were negative in all cases.

Total counts obtained at 20° C. were, in general, the maximum counts obtained for the samples plated. However, there were several exceptions. It should be emphasized that in four samples the total count at 20° C. was from ten to two hundred times more than the count obtained on the same sample at 37° C. A similar general relationship could be found for caseolytic and lipolytic counts at 20° C. In a majority of cases there were larger numbers of these organisms demonstrated at 20° C.

Incubation at 8° C. produced some variable results. So far as the data are concerned, there were fewer samples with complete data at 8° C. due to an accident. However, with the twelve samples on which considerable data were obtained, it may be observed that in only one case did the count at 37° C. exceed the total count at 8° C. Several samples showed counts at 8° C. greatly exceeding those obtained at 37° C. In fact, the counts at 8° C. in two cases were maximum for the temperatures used, and in one case, were significantly greater than those obtained at 20° C.

The differential counts at 8° C. did not follow the same relationship as those at the other temperatures used. In comparing the percentages of total numbers which were caseolytic and lipolytic at a given temperature, it may be seen that the temperature of incubation affected the lipolytic and caseolytic types more than the total count. In a majority of samples, the percentage of caseolytic and lipolytic organisms was greater at 20° C. and 8° C. than at 37° C.

The results obtained indicate that the various incubation temperatures exert a great influence on the number of organisms demonstrated in some samples. Even on the basis of the small number of samples plated, it appears that there is a trend for higher counts to appear at temperatures other than 37° C. This holds both for high and low count samples. Also, the flora measured and the balance of types is entirely different at the various temperatures. While the results are not complete so far as the 8° C. incubation is concerned, we may see that in relation to the maximum counts obtained, incubation at 8° C. generally revealed a considerable percentage of the organisms present, and at times might develop the maximum numbers capable of being counted.

II. *Bacterial counts on butter samples.* Data on the butter samples are presented in table 2. Again, the data are not complete for all samples because of the accidental loss of plates in five cases. The results obtained show that the first twelve samples gave very high counts when plates were incubated at 20° C. Likewise, there were considerable numbers of lipolytic and caseolytic organisms present. It may be observed also that there was no uniformity in the ratios of the lipolytic and/or the caseolytic to the total counts in the samples studied. As with the water samples, the flora of the butter samples depended on the individuality of each sample studied.

The data also show that there may be considerable numbers of organisms in butter which are capable of growing at 8° C. However, the same rela-

TABLE 2

Bacterial counts of butter samples

Sample	Incubation temperature	Total count	Proteolytic count	Lipolytic count
	°C.	*per ml.*	*per ml.*	*per ml.*
B1	20	10,000,000	est. 2,000,000	1,280,000
	8	x	x	x
B2	20	5,960,000	40,000	59,000
	8	x	x	x
B3	20	4,890,000	380,000	119,000
	8	x	x	x
B4	20	7,000,000	est. 2,000,000	320,000
	8	x	x	x
B5	20	4,860,000	430,000	22,000
	8	x	x	x
B6	20	2,170,000	40,000	6,000
	8	6,000	1,000	1,000
B7	20	2,670,000	9,000	83,000
	8	98,000	1,000	1,000
B8	20	2,530,000	270,000	70,000
	8	420,000	1,000	1,000
B9	20	60,000,000	TNTC	90,000
	8	1,310,000	1,000	1,000
B10	20	10,800,000	1,600,000	470,000
	8	1,950,000	1,000	1,000
B11	20	4,800,000	900,000	14,000
	8	630,000	1,000	1,000
B12	20	1,470,000	310,000	1,000
	8	88,000	1,000	1,000
B13	20	24,000	5,000	1,000
	8	1,000	1,000	1,000
B14	20	51,000	4,000	3,000
	8	1,000	1,000	1,000
B15	20	23,000	4,000	2,000
	8	3,000	1,000	1,000
B16	20	38,000	4,000	1,000
	8	1,000	1,000	1,000
B17	20	43,000	3,000	1,000
	8	8,000	1,000	1,000
B18	20	27,000	10,000	1,000
	8	5,000	1,000	1,000
B19	20	36,000	3,000	1,000
	8	1,000	1,000	1,000
B20	20	43,000	2,000	2,000
	8	1,000	1,000	1,000

(x) Plates discarded because of SO_2 leak in incubator. TNTC—too numerous to count in 1: 1,000,000 dilution.

tionships as are found with the water samples do not obtain. Nevertheless, the presence of cryophilic organisms in the butter samples has been conclusively demonstrated.

III. *Identification of the cultures isolated.* Forty-one cultures were isolated and identified as to genus. The results indicate that the isolated organisms were quite closely grouped, and the majority seemed to fall into one genus. Twenty-eight of the cultures were classified as belonging to the genus *Pseudomonas,* the majority of these cultures being able to produce a green fluorescence in culture media. Five cultures were placed in the genus *Flavobacterium* because of their ability to produce yellow pigment. Six cultures of gram-negative rods, showing little or no activity on culture media, were placed in the genus *Alcaligenes.* One culture was placed in the genus *Achromobacter.* One yeast culture was isolated and cultural characteristics determined, but no attempt was made to identify it.

The results of pasteurization survival studies have commercial importance since heat treatment of butter wash-water supplies has been advocated by a number of authors. Six of the forty-one cultures were able to survive exposure to 150° F. for 30 minutes. The surviving organisms showed considerable caseolytic activity.

A number of the cultures studied were able to produce an oxidase reaction of marked intensity. Castell and Garrard (7) have observed that most dairy products, showing defects caused by bacteria, have a flora extremely high in this type and that culpable water supplies likewise possess such organisms. The close relationship between these cultures and their ability to produce defects is emphasized by the results obtained in the incubation studies using sterile cream as a substrate.

IV. *Incubation studies using cream and skim milk.* All of the identified pure cultures were inoculated into sterile cream, followed by incubation both at 8° C. and 20° C. for the purpose of determining the type of defect produced from milk constituents. Repeated studies with these cultures indicated that odor production in cream both in type and intensity was quite constant under given conditions. The results showed that the temperature of incubation influenced the type of defect developed. The defects developed at 8° C. could be more specifically described and more closely resembled the typical butter defects than those at 20° C. The cream incubated at higher temperatures, in the majority of cases, became extremely putrid in a short time. The length of incubation period likewise played a part in the development of typical defects in that changes were constantly occurring within the culture as to the type and concentration of substances detected organoleptically.

A mixed sample of the worst cream cultures was compared with a sterile cream-control in attempting to develop a procedure whereby some of the volatile sulfur compounds might be measured both qualitatively and quantitatively. The distillates from the deteriorated cream were subjected to a number of qualitative tests for sulfur-containing compounds. The results of this work are presented in table 3. The distillate from the decomposed cream was without doubt different from that of the cream control. The data showed a stronger nitroprusside test, a stronger test for sulfides and sulfates (on oxidation) in the distillate from the decomposed cream. The distillate in the sterile control gave slight reactions to the nitroprusside test and the presence of sulfides and sulfates. This is to be expected because of the heat treatment of the cream prior to the incubation test.

The inoculation of five pure cultures into sterile skim milk and cream produced some interesting results. The cream and skim milk were compared to determine if the defect produced would be affected by the composition of the substrate used. The acidity and formol titration were run as an index of the extent of decomposition, and an attempt was made to correlate these results with the type and intensity of defect as judged organoleptically. The data given in table 4 indicate definitely that odor production is not dependent on the amount of protein breakdown as measured by the formol titration. In all five cultures it was found that the defects were much more pronounced and intense when cream was used as a substrate. The quantitative tests run on the cream cultures indicated that some sulfur compounds may be involved in the odor production. Trace amounts of mercaptans were found in two of the cultures. In addition, several of the cultures were able to produce small amounts of H_2S from the cream.

TABLE 3

Effect of bacteria on cream as measured by qualitative tests on the distillate

	Control	Decomposed cream
pH	6.0	8.9
Sodium nitro-prusside test	+	++
Mercaptans	−	−
Sulfates	+	++
Sulfides	+	+++

Another interesting observation during the course of the experiment was made on the distillates of the various cream cultures. It was found on completion of the distillation process that the distillates all had a common odor resembling heavily browned toast. The odor was one that would be associated with the heating of an organic mixture. However, it was not suggestive of severe burning or charring nor was it in any way typical of the culture distilled. On standing in the refrigerator for two or three days in a stoppered flask it was found that an odor appeared which closely resembled or was identical with that present in the original cream culture. No attempt was made to determine the reason for such an observation, but it was

TABLE 4

Effect of bacterial cultures on cream and skim milk
(After 10 days at 8° C.)

Culture	Medium	Acidity*	Formol*	Criticism	H_2S	Mercaptans
Control	Cream	1.5	1.7	Cooked	–	–
	Skim	2.3	2.8	Cooked	†	†
P28	Cream	3.3	3.0	Very putrid	–	–
	Skim	3.6	3.9	Sl. putrid	†	†
P70	Cream	3.7	2.5	Decaying cabbage	+	Trace
	Skim	3.2	6.5	Sl. putrid	†	†
P78	Cream	5.25	3.35	Putrid—limburger	–	Trace
	Skim	4.50	10.90	Sl. putrid	†	†
P84	Cream	4.1	3.3	Strong cooked corn	+	–
	Skim	3.5	6.9	Sl. cooked corn	†	†
L30	Cream	2.30	1.9	Putrid, caramel	+	–
	Skim	3.25	2.4	V. sl. putrid	†	†

* Results expressed in milliliters of N/10 NaOH to titrate 25 milliliters of the sample.
† No determinations made.

thought to be due to oxidation-reduction processes which might go on during and after the distillation procedure.

DISCUSSION

The results obtained on the counts of both water and butter indicate that there is a portion of the flora in these samples which may remain unmeasured when using mesophilic incubation temperatures. The results obtained when using 37° C. incubation and the presumptive coliform test with brilliant green-lactose-bile broth indicate the inadequacy of public health methods of analysis for creamery water supplies. The potability of most of the waters would scarcely be questioned, but the unsuitability of several of them for creamery work has been demonstrated. A satisfactory water supply is one which is relatively free from all bacteria and particularly those types which are able to attack milk constituents at low temperatures. Therefore, it is imperative to use methods which will measure this flora adequately.

The fact that butter may contain a large number of cryophilic organisms is not surprising, for frequently storage at temperatures just above freezing serves as an "enrichment" process for the growth of these types at the expense of others requiring higher temperatures. Contamination of water is not so important from the quantitative as from the qualitative standpoint. However, in the past, the magnitude of count has been constantly emphasized, whereas a low count water may have been considered satisfactory and unimportant so far as dairy products were concerned. It should be recognized, however, that such a water may be a source of few but very important organisms of the lipolytic and caseolytic types, which may be able to grow at low temperatures.

A majority of the isolated cultures showed marked lipolytic and/or caseolytic action, which points to the possibility of their ability to cause trouble. The observation that some samples contained a large number of cryophiles which were inert on differential media does not decrease the significance of the count. When using skim milk agar, the measurement of caseolysis may be a function of temperature as has been noted by Sarkaria and Hammer (33). Simply because an organism does not produce any visible change on casein at low temperatures does not mean that it is harmless to butter keeping quality. It has already been demonstrated that intensive odor production may occur in cream without extensive proteolysis. There are a number of the minor constituents of milk which are proteins or protein-like in nature and which may be attacked by bacteria.

The development of more typical and intense odors in cream as compared with skim milk is an indication that the deterioration may be intimately connected with the fat. In this connection, there appear to be several possibilities. The pure triglyceride itself may be responsible or some substance associated with it—the phospholipids or the fat globule membrane. However, the distillation studies indicate that the material involved is a protein or protein-like in nature since the sulfur in the medium is one of the constituents affected. It would, therefore, seem that the protein-like materials in the fat globule membrane and the phospholipid fraction are most likely involved. In addition, the odor defects produced are suggestive of a proteolytic change.

SUMMARY

1. In plating out creamery water samples and butter samples on two differential media, it was found that 20° C. incubation gave, in most cases, the highest counts. However, 8° C. incubation yielded counts that were of considerable magnitude. In no sample did 37° C. incubation produce maximum total counts.

2. Caseolytic and lipolytic cultures capable of vigorous growth at 8° C. were isolated and purified. Of the forty-one cultures studied, twenty-eight belonged to the genus *Pseudomonas*, five to the genus *Flavobacterium*, six were species of *Alcaligenes*, one was classified as an *Achromobacter*, and one was a non-lactose-fermenting yeast.

3. Incubation studies using sterile cream inoculated with pure cultures showed that a large majority of these cultures were capable of causing extensive deterioration as measured by odor production. The production of odor defects typical of each culture depended on the temperature and the time of incubation.

4. The effect of five pure cultures on cream and skim milk was observed organoleptically and measured by acidity and formol titration. It was found that the intensity of the odor production was not correlated with extensive protein deterioration, as measured by the formol titration. It

was also observed that odor production was more intense in cream than in skim milk. Positive qualitative tests for hydrogen sulfide and the presence of mercaptans were obtained during the process of distillation of several of the cultured cream samples.

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MEASURING DETERGENCY FUNCTIONS AS AFFECTED BY VARIOUS DETERGENTS AND PROCEDURES AGAINST MILK FILMS BY APPLICATION OF A MECHANICAL WASHING APPARATUS*

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The detergency qualities of washing powders have in most instances been determined by theoretical attributes based on chemical constituents and reactions. However, investigators have also used mechanical washing devices to measure the efficiency of detergents. Schwartz and Gilmore (12) studied detergency from glass dishes of a prepared soil analogous to that normally formed from food sources, using a standard mechanical dishwasher and presented averages of light transmission readings obtained by use of a Zeiss photometer.

Gilcrease and O'Brien (4) developed a functional test, involving washing apparatus for microscopic slides and used a photoelectric colorimeter to evaluate various products marketed for dishwashing. A standard soil of similar constituency to that frequently encountered in dishwashing was also used by these investigators for studying detergent efficiency.

Scales and Kemp (10) prepared sheets of metal with an adhesive milk film by exposing 3-inch squares of tinned copper to milk just below the boiling point. After drying 4 days at room temperature the metal squares were washed by dipping in washing solution at 120° F. for one minute without agitation after which the slides were moved back and forth for one minute to create circulation of the solution across the surface.

Numerous investigations on detergency of various dairy washing products have been made. Alkaline powders have been generally accepted and detergency factors applying to these products have been extensively reported. Generally alkaline products have been evaluated for detergency qualities on the basis of pH and sodium oxide content, and for qualities of water softening, wetting, dispersing and emulsifying action. An excellent review of early work on alkaline detergents has been done by Barnum, Lucas and Hartsuch (2). However, alkaline detergents have not been completely satisfactory for dairy utensil washing. Sommer (13) reports that sodium hydroxide and carbonate washing powders cause granular precipitates that tend to cling to the surface of equipment; while Parker and Johnson (7)

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associate alkaline detergents with formation of milk stone on equipment. Also alkaline detergents have not been found suitable for flush-washing cream separators due to causing excessive sliming within the separator bowl (5).

The use of acid detergents for the dairy industry was first reported in 1940 by Scales and Kemp (11). Parker (8) confirmed excellent cleaning obtained with acid.

Cleaning trials with synthetic organic detergents were first reported by Scales in 1939 (10). Rapid application has since been made of these products in conjunction with alkaline and acid detergents in the dairy industry.

Sodium hexametaphosphate and sodium tetraphosphate have been used industrially (9, 12) as mineral sequestering agents, and have mainly been used in detergency in combination with alkaline powders to inhibit calcium reaction by tying up the calcium ion and thus preventing the formation of calcium precipitation of casein or carbonate.

A neutral or near-neutral detergent in the nature of a *wetting agent* and metaphosphate was found by the author (5) to effect improved detergency over a wetting agent detergent alone for flush-washing cream separators.

The condition of the water with respect to detergency has received attention. Water-softening has been a recognized requirement of alkaline washing powders; while wetting agent detergents have also been shown to effect mineral precipitation in hard water when used in the quantity required for detergency (5, 6).

In view of the many types of dairy detergents that are available, and in consideration of the difficulty in maintaining utensils clean and free of milk and mineral residues, it seemed desirable to apply laboratory washing tests to determine comparative washing performance and conditions under which several of these products could be used most effectively.

PROCEDURE

Under practical washing conditions glass surfaces were repeatedly coated with raw milk films and after each washing treatment the glass was examined for light transmission to determine residual filming by means of a Cenco-Sheard Spectrophotelometer.

Five detergents of different type were selected for use to determine their detergency under varying conditions of use.

Washing trials were made to measure the detergency obtained when the sequence in washing technique involved:

(a) Prerinse, wash, after-rinse.
(b) No prerinse, wash, after-rinse.
(c) Washing only.
(d) Prerinsing with water of different hardness.
(e) After-rinse using soft water.

(f) After-rinse using water treated with sodium metaphosphate and sodium metaphosphate–wetting agent detergent.

Unetched, clean, 3-inch squares of glass were coated with raw milk films by twice immersing in well-mixed raw whole milk held at 95° F. The coated slides were placed on a frame at approximately 45° angle to permit draining and drying for a period of 5 to 10 minutes before washing.

Washing was accomplished by use of a motor-impelled bar to which the glass squares were attached in a manner that each was washed in a separate water bath containing 1500 ml. of different washing solutions. Description of this apparatus is shown in figure 1.

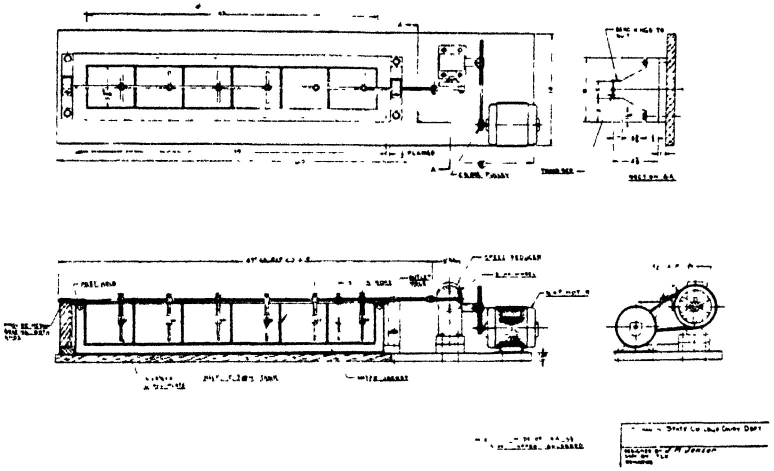

FIG. 1. Diagrammatic drawing of washing apparatus for glass or metal discs. The author is grateful to Professor A. H. Farrall, of the Department of Agricultural Engineering, for the drawing of the washing apparatus.

The glass squares were propelled at a rate of 45 oscillations per minute, and for one minute washing time preceded by one minute of soaking similar to procedure used by Scales and Kemp (10).

Washing temperatures used were, 95° F. for pre-rinsing, 120° F. for washing and 150° F. for after-rinsing. Prerinsing consisted of impelling the milk-coated glass slides 5 complete cycles through the rinse water at a distance of 3 inches in a manner to force the water across the face of the glass. Rinsing after washing also consisted of 5 complete cycles.

After the glass slides were dried at room temperature, light transmission was measured. Four readings were obtained from each square by placing the corners in the filter position in the spectrophotelometer. Readings were made with adjustment of the entrance slit at 1 mm., exit slit at 20 mm., and 400-mm. wave length. The galvanometer was adjusted for zero reading with no light transmitted, and for 100 with light transmitted through a clean glass of the same quality as that used for the washing trials.

The pH of washing solutions was determined by use of a Beckman pH meter. No other chemical analyses were made of the detergents used.

The tap water used in the washing and rinsing processes contained 300–320 p.p.m. of hardness as determined by soap titration (1), and the zeolite-softened water contained less than 30 p.p.m. hardness. Metaphosphate treated pre-rinse water, as used in figure 4 was determined by calculation based on a requirement of 1.25 g. sodium metaphosphate per grain of hardness in 100 gallons of water (5).

RESULTS

The pH of solutions of detergents used. Five proprietary detergents, representing those most commonly found among dairy farm utensil cleaners were used in this study. They include (a) an alkali with wetting agent; (b) an alkali with neutral polyphosphate salt; (c) an acid cleaner; (d) a wetting agent and (e) a near neutral polyphosphate salt with a wetting agent. The specific product used for each group was selected without previous evaluation as to its detergency value. Therefore, it is entirely possible that the product selected for study was not necessarily the best in the group. However, it is believed that these are representative of the several types of cleaners available for dairy utensil washing.

TABLE 1

Description of detergency products used in washing trials

Designation of product	pH of 0.25%* solution at 77° F.	Alkaline salts	Neutral polyphosphate salts	Wetting agent†	Acid
		per cent	*per cent*	*per cent*	*per cent*
A	9.87	97		3	
B	9.45	80	20		
C	2.00			Not known	Not known
D	8.00			100	
E	6.50		75	25	

* 0.8 per cent solution and 0.15 per cent were used for C and D, respectively.

† Wetting agent powders used contain approximately 60 per cent sodium sulphate. This is included as wetting agent in quantity listed.

The description of the detergents used, which have been designated as A, B, C, D, and E, is presented in table 1. Trials indicated that the pH of a 0.25 per cent solution ranged from a high pH of 9.87 to a low of pH 2.0. Hence, these detergents possess a wide range in reaction which may have an important bearing on their detergency value when used in connection with milk films, the product with which the dairymen must reckon in all dairy utensil cleaning.

Effectiveness of various types of detergent in removing milk films. Glass squares were coated with milk film and washed under controlled conditions, as explained under procedure, repeatedly 14 days using the various types of detergent described in table 1. The specific data by days are presented in figure 2 whereas averages are presented in figure 3. From figure 2 it will be noted that the most effective detergency occurred under all conditions of washing when detergent E, the near-neutral mixture of wetting agent and metaphosphate, was used.

The removal of milk film with detergent D, the slightly alkaline wetting agent, was more complete than with either of the strictly alkaline or acid detergents. However, the results secured when employing the usual washing procedure of prerinsing, followed by solution washing and rinsing with hot tap water afterward, were no better than when a similar procedure was used with alkaline detergent A. An improvement resulted when a washing procedure that differed from the usual practice was used, as is shown later in this article.

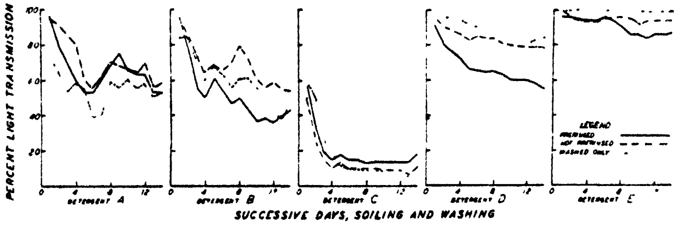

FIG. 2. Effect of repeated washing of milk-coated glass with five different detergents in tap water at 300 to 320 p.p.m. hardness. Washing procedures included (a) prerinsing using 95° F. water, washing with 120° F. solution and after rinsing with 150° F. water; (b) washing with 120° F. solution and rinsing with 150° F. water with no prerinse; (c) washed only using 120° F. solution without prerinsing or after-rinsing.

Acid detergent C had inferior detergency qualities and appeared to cause protein precipitation on the glass surface. The poor detergency could possibly be accounted for by the low washing temperature (120° F.) and low pH at which these washing trials were conducted, although the amount and temperature directed by the manufacturer were used.

Filming on the glass did not always develop with regular intensity, but as measured by spectrophotometer reading, showed occasional recovery in light transmission. The alkaline detergents A and B, figure 2, gave rise to greater fluctuation in spectrophotometer readings than the other detergents used. This variation in filming gave evidence of detergency properties that were inadequate to remove the inner and more tenaciously bonded films; but sufficient to remove the outer and more loosely held accretions.

The superior washing results that were secured with near-neutral detergent E definitely indicated that detergency of milk films was not contingent upon chemical reaction of alkalinity or acidity, but on basic properties of wetting, emulsifying and dispersing. A properly combined compound con-

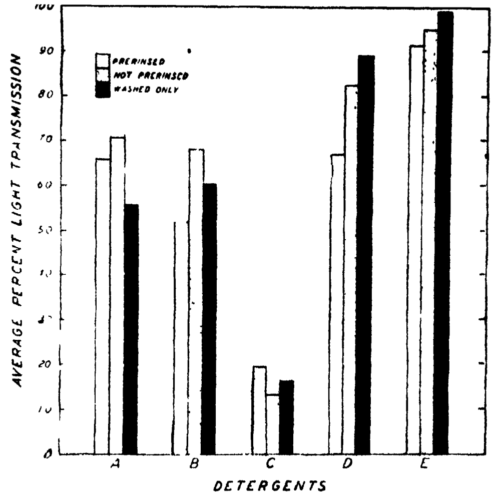

FIG. 3. Average per cent light transmission of 14 repeated washing trials when 5 different detergents were used in tap water at 300 to 320 p.p.m. hardness. The washing procedures included (a) *prerinsing*, washing and after-rinsing; (b) washing, after-rinsing, but not prerinsed; (c) washing only without prerinsing or after-rinsing.

sisting of sufficient wetting agent for fat emulsification and wetting action, plus a near-neutral polyphosphate to perform the function of dispersing the milk solids, while also suppressing mineral salt precipitation, appeared to harmonize in a manner to effect superior detergency on raw milk films. Although detergency qualities were possessed by the alkaline and acid products used in these trials, it was altogether probable that alkalinity or acidity adversely affected the adhering condition of milk protein. At least the high detergency secured with the slightly alkaline product D and the near-neutral product E would indicate that products possessing physical detergency qualities (11) rather than qualities depending upon chemical reaction were most suitable for raw milk film removal.

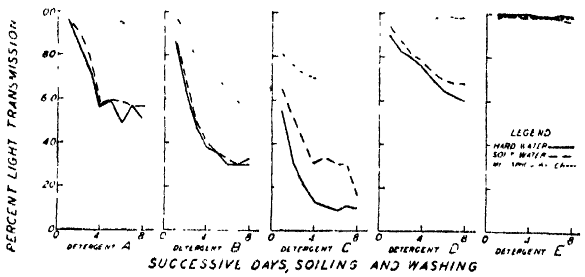

FIG. 4. The effect on light transmission when the quality of prerinse water was varied to include (a) hard water at 300 to 320 p.p.m. hardness; (b) zeolite-softened water at less than 20 p.p.m. hardness; and (c) metaphosphate water treated in excess of 0 hardness.

Influence of prerinsing on removal of milk film. In the series of 14 washings made wherein the milk-coated glass squares were washed without prerinsing, outstanding improvement resulted with all detergents except C, as shown by figures 2, 3, 5 and 6. Especially outstanding was the improvement evident with products B and D.

These results were of special interest inasmuch as prerinsing has been considered a most necessary function under all washing conditions to prevent the forming of milkstone as well as to preserve the quality of the washing solution.

As a means of determining whether the hardness or the condition of the prerinsing water influenced the difference in film adherence, prerinsing was conducted in hard water, soft water and hard water treated with excess sodium metaphosphate, in sufficient quantity to account for 350 p.p.m. hardness in 300–320 p.p.m. water. Since sodium metaphosphate has not been considered a detergent, the difference obtained in detergency value when metaphosphate prerinse was used could be regarded as being attributed to a change in the nature of the milk film due to the action of metaphosphate on the milk bound calcium. The influence of these conditions is presented in figure 4, and shows that film removal was only slightly improved when soft water was used for prerinsing over hard water with all detergents except C where substantial improvement occurred. The influence of excess metaphosphate solution for prerinsing resulted in exceptionally high improvement in detergency for all products except E. Since product E gave practically complete film removal under all prerinsing conditions this product could hardly be improved. However, detergent D gave similarly high spectro-photometer readings when prerinsing with the metaphosphate solution.

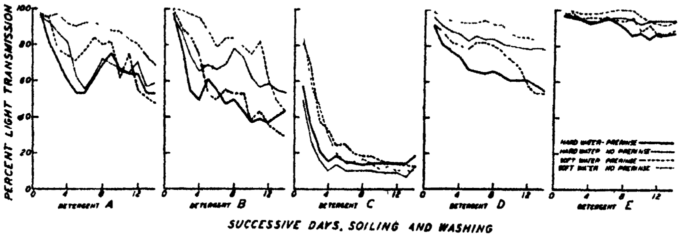

FIG. 5. Effect of hardness of water used for the final rinse on light transmission when milk-coated glass slides were repeatedly washed in 5 different detergent solutions, with and without prerinsing. Hard water at 300 to 320 p.p.m. and zeolite-softened water at less than 30 p.p.m. were used for final rinse.

These results substantiate the soundness of washing certain utensils like cream separators and milking machines with wetting agent detergents by flush washing procedures without prerinsing except as required to flush excess cream from separator parts (3).

It appeared likely that untreated water reacted with milk constituents as a result of the prerinse to increase the fixing quality of the milk film. Since detergency was highly improved with metaphosphate added to the rinsing water it was conjectured that the film fixation quality of normal milk and water was related to the chemical and physical behavior of calcium and casein.

A practical application of prerinsing with near-neutral polyphosphate salt solutions could possibly be developed for better results with mechanical washing of milk cans.

Influence of detergency when washed without prerinse and after-rinse. Washing trials were also conducted to determine the influence of not rinsing after washing on the cleanliness obtained on glass slides washed with the various detergents. These results are also presented in figures 2 and 3.

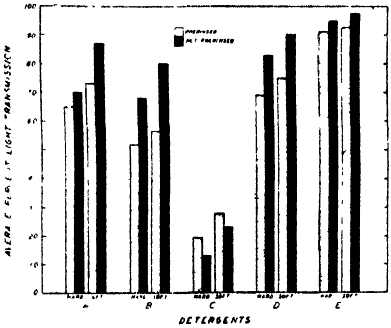

FIG. 6. The effect of hardness of water used for the final rinse on the average light transmission readings, obtained from 14 successive washing trials with and without prerinsing.

Oddly enough highest detergency was secured for washing products D and E, slightly alkaline and near-neutral detergents respectively, when no rinsing treatment was applied either before or after washing. The acid detergent also indicated slight improvement when not rinsed following the washing treatment. The alkaline detergents, however, gave materially improved detergency values when a final rinse with clear water at 150° F. was applied.

Influence of soft water in final rinse on washing results. Washing was conducted employing soft water for the final rinse under conditions wherein prerinsing was used on one set of slides, but not on the other. To simplify a comparison of the results when rinsing with soft water and hard water under similar washing conditions, the results are presented in figures 5 and 6. The *hard water* washing data being the same as shown in figures 2 and 3. Fairly high improvement was shown in favor of soft water for rinsing after washing for all the detergents, but only scant improvement again was possible for detergent E, because of its high detergency value when hard water

TABLE 2

Effect of sodium metaphosphate in final rinse water at 150° F. on light transmission of glass slides when washed with neutral metaphosphate–wetting agent detergent at 0.25% concentration. No pre-rinse

Per cent sodium metaphosphate salt in rinse solution	Per cent light transmission									
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
0.05	87	93	92	98	98	97	95	96	95	96
0.10	97	96	95	97	96	96	95	95	95	95
0.15	98	96	96	96	97	97	98	96	96	97

* Average of two trials.

was used. Attention is also again called to the high improvement in detergency when no prerinse was used. Under such a washing condition products A and D gave highly improved detergency.

Effect of adjusting final rinse with sodium metaphosphate and sodium metaphosphate plus wetting agent. The results obtained when glass slides were washed with a sodium metaphosphate–wetting agent mixture and not rinsed, as shown in table 2, tended to indicate that the remaining surface retained a protective layer to which the milk film did not adhere as firmly as on a surface rinsed free of such a film. Since rinsing after washing with hot water is necessary as a sanitizing measure, it was considered feasible that better detergency conditions could be maintained, providing the rinsing water contained a certain amount of metaphosphate or possibly metaphosphate–wetting agent detergent. Trials were, therefore, made on washing milk-coated films with neutral sodium phosphate–wetting agent detergent E at 120° F. and rinsing with 150° F. water containing 0.05, 0.10 and 0.15 per cent, respectively, of sodium metaphosphate in one trial and the same quantities of detergent E in another. The results are shown in tables 2 and 3.

Results in table 2 show that when the final rinsing water contained sodium metaphosphate in percentages of 0.10 and 0.15 consistently high light transmission was obtained, with some increase in this respect favoring the higher quantity used. When only 0.05 per cent was used the rinsing solution

TABLE 3

Effect of sodium metaphosphate-wetting agent detergent in final rinse water at 150° F. on light transmission of glass slides when washed with similar detergent at 0.25% concentration. No pre-rinse

Per cent sodium metaphosphate–wetting agent detergent in rinse solution	Per cent light transmission									
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
0.05	90	95	89	83	89	89	88	89	88	87
0.10	99	99	99	98	97	97	98	97	98	97
0.15	99	100	100	100	100	100	99	100	100	99

appeared slightly turbid, indicating an unstable calcium condition. Although this condition occurred in all solutions the first washing was the most measurably affected. Two trials were made to verify this condition and the results presented are their averages.

When detergent E was used at 0.10 and 0.15 per cent levels in the rinsing water higher light transmissions were observed than when sodium metaphosphate at these percentages were used. Also the 0.15 per cent quantity gave higher light transmission and slightly better results than when 0.10 per cent was used.

When only 0.05 per cent of detergent E was added to the final rinsing water turbidity occurred, along with lower light transmission. This condition was apparently due to an insufficient quantity of metaphosphate to inhibit calcium reaction with the sodium sulphate accompanying the wetting agent.

Consistently high light transmission observed adequate cleaning when quantities of detergent E were used in rinsing water again suggested that a protecting film of wetting agent and sodium metaphosphate adhered to the surface and prevented the more tenacious bond otherwise formed by milk.

SUMMARY

Detergency studies were made on various types of washing products. These were made by washing raw milk films from glass surfaces by a devised mechanical apparatus and measuring result in terms of light transmission by use of a Cenco-Sheard spectrophotelometer.

An appreciable difference was observed in detergency as affected by detergent material and variations in washing procedure that was measured by light transmission readings. Highest detergency values were secured with solutions prepared of near-neutral wetting agent and metaphosphate mixtures. Next in order of detergency were slightly alkaline wetting agent detergent, alkaline salts containing wetting agent, alkaline salts containing metaphosphate and acid detergent solution which gave lowest and entirely inadequate detergency.

Prerinsing effects demonstrated depressive detergency action with both hard and soft water with all detergents except acid. Better detergency was secured with soft water than with hard water. Highly improved detergency was attained when prerinsing with water containing metaphosphate in slight excess of that required for sequestration of water hardness.

After rinsing effects demonstrated improved detergency with alkaline detergents when hard and soft water were used at 150° F. Depressed detergency occurred with acid, wetting agent and wetting agent plus metaphosphate detergents. However, soft water for final rinse gave better results than hard water.

When wetting agent–metaphosphate detergent was used and the final rinse contained 0.10 and 0.15 per cent metaphosphate in water containing 300 to 320 p.p.m. hardness, high light-transmission readings were maintained throughout repeated washing trials. Highest and practically complete detergency as measured by light transmission occurred when 0.10 and 0.15 per cent wetting agent–metaphosphate detergents were usd in the final rinse.

ACKNOWLEDGMENT

The author wishes to acknowledge assistance rendered by Mr. Robert Frantz in conducting laboratory washing and making instrument readings of results.

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THE RELATIVE IMPORTANCE OF HIGH TEMPERATURE AND HIGH HUMIDITY AS FACTORS INFLUENCING RESPIRATION RATE, BODY TEMPERATURE, AND PULSE RATE OF DAIRY COWS

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Results of various studies (5, 7, 8, 11) have focused attention on the importance of summer climatic conditions as factors affecting the production of dairy cattle. Experimental results have shown, in general, a reduction of the fat percentage of the milk and, in some cases, a decrease in the quantity of milk produced. Two primary reasons are advanced (9, 10, 11, 12) for decreases in production during summer months. One is the lack of an abundant supply of green succulent milk-producing feed such as is supplied by an excellent pasture. The second is the inability of the cow to dissipate heat at a fast enough rate (2, 6) to keep up with the heat-producing effect of existing climatic conditions. As a result, various studies (3, 8, 9, 10, 11, 12) have shown that cows develop greatly accelerated respiration rates and higher body temperatures. One recently reported study of Jersey cows in Louisiana (4) showed correlations between air temperature and respiration rate of 0.77 and between air temperature and body temperature of 0.57. When air temperatures were below 70° F. the correlation was insignificant.

Reports on two studies (1, 10) show that high humidity, in addition to high temperature, may be an important factor in causing lower production and increases in respiration rate and in body temperature. The data are rather scanty, however, and this was one reason why the present investigation was initiated. Louisiana conditions in summer are such that relatively wide variations occur in both temperature and humidity, thus affording a good environment for testing the relative importance of these two climatic factors in causing changes in respiration rate, pulse rate and body temperature of dairy cows.

PROCEDURE

Data used in the present study were secured from milking cows of the Louisiana State University dairy herd. The cows were handled in two separate dairy units and each unit included both Jersey and Holstein cows. In 1944 there were 36 Holsteins and 16 Jerseys, while in 1945 observations were made on 41 Holsteins and 27 Jerseys.

Respiration rates were secured from counts made of flank movements and body temperatures from readings made with veterinary thermometers inserted approximately three inches into the rectum. Pulse rates were secured

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by placing tips of fingers next to coccygeal arteries on under side of tail. In most cases respiration and pulse rates were checked by two people and when there was a lack of agreement a third count was made. These records were secured from 10 to 30 minutes after the cows entered the milking barn (at approximately 3:00 P.M.) and before they were fed grain.

During 1944 records were secured on consecutive days twice weekly, starting on August 28 and ending on October 14. In 1945 each herd was observed on 15 different days, with these days falling between July 16 and August 3 for one herd and between August 6 and August 24 for the other.

A sling psychrometer was used to secure wet- and dry-bulb temperature records at the time of each observation. Appropriate standard weather bureau tables were used to determine relative humidity from the readings secured.

RESULTS

The atmospheric temperature and relative humidity prevailing during the periods of observation showed fluctuations as indicated in table 1. Response of the milking cows to these atmospheric conditions is also shown and their body reactions indicate relatively large increases from those considered normal, 101.1° F. (4) for body temperature, and 22 for respirations per minute. This would indicate that the atmospheric conditions prevailing during the course of the experiment departed sufficiently from normal to demonstrate just what effect variations in summer atmospheric conditions have on body temperature, respiration rate, and pulse rate of dairy cows.

TABLE 1

Summary of air temperature, relative humidity, body temperature, respiration rate, and pulse rate for periods covered in the experiment

	Periods of observation					
	1944			1945		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Air temperature (°F.)	65–93	85.1	6.17	75–91	85.6	4.75
Relative humidity (%)	27–91	56.8	14.06	51–91	73.5	10.00
Body (rectal) temperature (°F.)						
Holsteins	100.2–106.5	103.31	1.27	100.0–106.0	103.46	1.22
Jerseys	100.3–105.6	102.60		99.0–105.9	102.71	
Respirations per minute						
Holsteins	21–129	67.4	24.0	28–129	80.4	20.2
Jerseys	22–128	69.9		23–143	75.9	
Pulse rate per minute						
Holsteins	40–91	67.7	6.66	32–94	70.6	7.22
Jerseys	50–99	65.9		53–99	70.0	

The analysis of covariance, multiple correlation, and partial correlation (13) were utilized for the analysis of the data secured in this study. It was first necessary to use covariance to remove from the data the variation due to differences between the two herds caused by the plan of handling the University cattle as two dairy units. This was found to be an important source of variation for both years. Next, differences between breeds within the herds were removed from the data. For body temperature this was an important source of variations as is indicated from the averages given for the two breeds, as shown in table 1. Respiration and pulse rate differences between breeds were only slightly important. After removing variance in data due to herd and breed differences, the final measurements in terms of correlation coefficients and regression equations are on an intra-herd and intra-breed basis and are thus appropriate for predicting what would happen in an individual dairy herd consisting of just one breed of cattle.

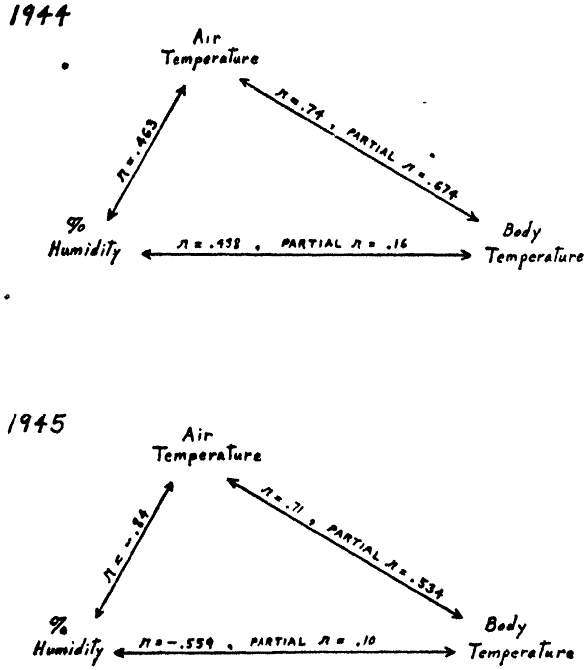

FIG. 1. Correlations between air temperature, relative humidity, and body temperature of cows on an intra-herd and intra-breed basis.

Body temperature. Relationships between air temperature, relative humidity, and body temperature on an intra-herd and intra-breed basis can be seen best by examining figure 1. This diagrammatic presentation indicates by correlation coefficients the relationship between the three variables. Of special interest is the positive correlation (r) between air temperature and relative humidity in 1944 and the negative one between these same two variables for 1945. The reversal in this relationship is attributable to sampling variation caused by the choice of days used for observation during the two years.

The correlation coefficients of 0.74 and 0.71 between air temperature and body temperature for the two respective years are relatively high and surprisingly alike, in view of the reversal in behavior of relative humidity in relation to air temperature for the two years. The correlation between relative humidity and body temperature, on the other hand, takes a reversal in form, being 0.438 for 1944 and -0.559 for 1945.

Using partial correlation, whereby the effect of one variable can be held constant, helped to remove the apparent discrepancy from the results secured. When this was done and humidity was held constant, the correlation between air temperature and body temperature became 0.674 for 1944 and 0.534 for 1945 (fig. 1). On the other hand, when air temperature was held constant and the relationship between relative humidity and body temperature was measured the correlation coefficient was 0.16 for 1944 and 0.10 for 1945.

By the use of multiple correlation it was found that the R for air temperature and relative humidity times body temperature was 0.748 for 1944 and 0.713 for 1945. These are only a little larger than the simple correlations between air temperature and body temperature. (See figure 1.) The multiple regression equations, having X represent air temperature and Y per cent humidity, were as follows:

For 1944, estimated body temperature $= 0.1364\,X + 0.0099\,Y - 9.08$

For 1945, estimated body temperature $= 0.1988\,X + 0.0129\,Y - 14.80$

An examination of these regression coefficients shows that those for air temperature are 13 and 15 times as large as are those for per cent humidity. This indicates that every degree increase in air temperature was responsible for from 13 to 15 times as much increase in body temperature as was caused by one per cent increase in relative humidity.

Respiration rate. Figure 2 presents the relationships found between respiration rate, air temperature, and relative humidity. As for body temperature, the relationships between the two years are somewhat confusing because of the reversal in the correlation between air temperature and humidity. Partial correlation coefficients for air temperature and respiration with humidity held constant were 0.748 and 0.353 for the respective years. Only the one for 1945 is appreciably changed from the simple correlations. With air temperature held constant the partial correlation between per cent humidity and respiration rate was -0.06 for 1944 and -0.02 for 1945. Although these coefficients are small they indicate a tendency for respiration to slow up slightly as per cent humidity increases.

Multiple correlation computations of air temperature and per cent humidity times respiration rate gave values for R of 0.778 for 1944 and 0.591 for 1945. Only the one for 1944 is changed at all from the simple r values shown in figure 2 and that one only slightly. This would indicate that the prediction value of a simple regression equation using changes in air temperature only, would be about as accurate as would a multiple regression equation which considers changes in both air temperature and per cent humidity. A consideration of these multiple regression equations with X

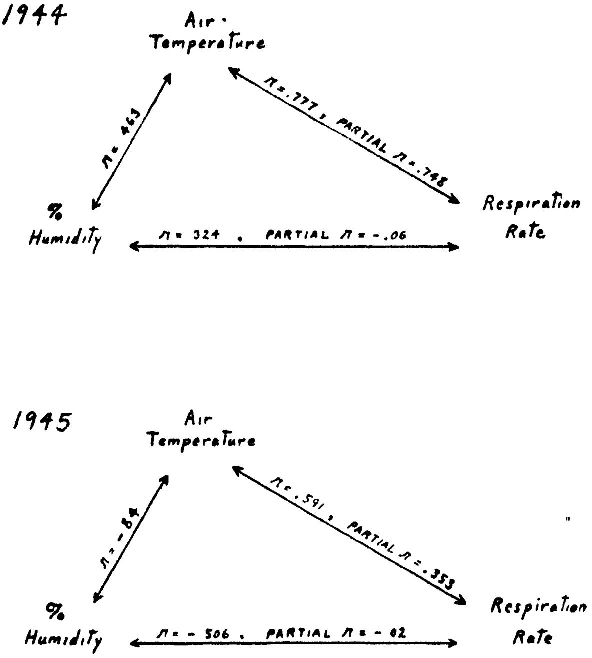
1944
Air-Temperature
r = 463
r = .777, PARTIAL r = .748
% Humidity
r = 324 , PARTIAL r = -.06
Respiration Rate
1945
Air Temperature
r = - 84
r = .591, PARTIAL r = .353
% Humidity
r = - 506 , PARTIAL r = - 02
Respiration Rate

FIG. 2. Correlations between air temperature, relative humidity, and respiration rate of cows on an intra herd and intra breed basis.

representing air temperature and Y per cent humidity will also show this to be true:

For 1944, estimated respiration rate $= 3.209\ X - 0.0752\ Y - 202.8$

For 1945, " " " $= 2.424\ X - 0.0585\ Y - 123.4$

The ratio between the respective coefficients shows that one degree change in air temperature was responsible for from 41 to 43 times as much change in respiration rate as was one per cent change in humidity. Tests of significance showed that the regression coefficient for humidity in 1945 was not sufficiently large to fall within the 5 per cent level of significance. This shows that a simple regression equation considering changes in air temperature only would be appropriate for estimating respiration rates for that year.

Pulse rate. Changes in air temperature apparently had a relatively small effect on pulse rates. Both the simple correlations and partial correlations (fig. 3) are positive and average slightly less than 0.2. On the other hand, the simple correlation of 0.05 between per cent humidity and pulse rate for 1944 became – 0.05 on a partial correlation basis with air temperature held constant. In reverse order, the simple *r* between these same variables for 1944 of – 0.114 became positive, 0.07, on a partial correlation basis. This reversal in relationship makes it impossible to draw any conclusion, other than to say that changes in humidity had only a small effect in either increasing or lowering pulse rates.

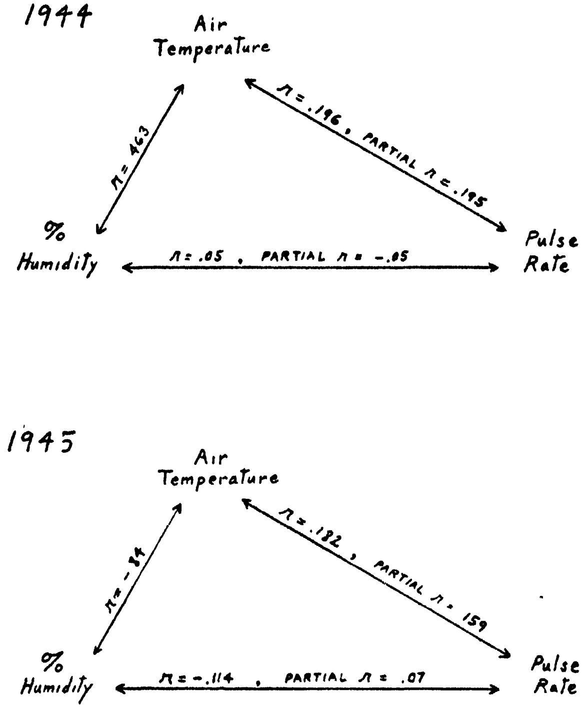
1944
Air Temperature
r = .463
r = .196, PARTIAL r = .195
% Humidity
r = .05 , PARTIAL r = -.05
Pulse Rate
1945
Air Temperature
r = - 84
r = .182 , PARTIAL r = .159
% Humidity
r = -.114 , PARTIAL r = .07
Pulse Rate

FIG. 3. Correlations between air temperature, relative humidity, and pulse rate of milking cows on an intra-herd and intra-breed basis.

DISCUSSION

Results of this study would indicate that relative humidity plays a minor rôle in comparison to air temperature as a factor influencing body temperature, respiration rate, and pulse rate. In the present study as contrasted to previous ones (1, 10) the effect of humidity was measured by partial correlation, which resulted in a statistic computed on a basis of air temperature held constant. The fact that the data for the two years yielded similar results is rather significant, in view of the fact that during 1944 there was a high positive correlation between air temperature and relative humidity, while in 1945 a high negative correlation existed between these two variables.

The small negative partial correlations (– 0.06 and – 0.02) between per cent humidity and respiration are in contrast with the small positive ones (0.16 and 0.10) between humidity and body temperature. It is possible that the slight tendency for an increase in humidity to slow up respiration was responsible for decreasing the rate of heat elimination from the cow's body, thus causing a tendency toward increased body temperature with the rise in per cent humidity. On the other hand, the tendency for increases in humidity to slightly slow up rate of respiration may be of real benefit to the cow. Regardless of the explanation, the present study tends to show that an increase in air temperature is many times more important than is an increase in relative humidity in causing changes in either body temperature or respiration rate. To a lesser degree, the same is true for changes in pulse rate, and in this case the independent effect of humidity gave conflicting results, as shown by a small positive partial correlation for one year and a small negative one for the other.

SUMMARY

Observations made of 36 Holstein and 16 Jersey milking cows during the summer of 1944 and of 41 Holsteins and 27 Jerseys in 1945 revealed the following:

1. Changes in air temperature appeared to be the major cause of increases in body temperature and respiration rate of milking cows.

2. Multiple regression equations indicate that one degree increase in air temperature was responsible for from 13 to 15 times as much increase in body temperature as was an increase of one per cent in humidity.

3. Likewise, one degree change in air temperature caused from 41 to 43 times as much influence on respiration rate as did one per cent change in humidity. On a partial correlation basis (air temperature held constant) an increase in humidity slightly lowered respiration rate. The *r* values were – 0.06 and – 0.02.

4. Partial correlations between air temperature and body temperature (humidity held constant) were 0.674 and 0.534.

5. Pulse rates were less affected by either air temperature or relative humidity than was body temperature. Air temperature and pulse rate measured independently of humidity gave *r* values of 0.195 and 0.159. Humidity and pulse rate (with air temperature constant) gave a value of – 0.05 for one year and 0.07 for the other, thus leaving a question as to its real relationship.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of members of the

Department of Dairying for the use of cattle used in this experiment. They also acknowledge assistance given by Dr. L. L. Rusoff of the Dairy Research Department in gathering some of the data and for constructive criticism of the manuscript.

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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-FIRST ANNUAL MEETING

EXTENSION SECTION

D.H.I.A. WORK

E1. Considerations in the Development of the Dairy Herd Improvement Association Program. KENDRICK, J. F., Bureau of Dairy Industry, U.S.D.A.

The need for more efficient producing dairy cows is greater today than ever before. More people are using more milk and milk products in their daily diets than ever before. All indications are that the present high demand for milk will not only continue, but that it will actually increase in the future. The human population, consumers of milk, is increasing. The dairy cow population remains about the same, actually decreasing during the last year.

More milk must be produced per cow; that is, the producing efficiency of the dairy herds of the Nation must be increased if the total milk supply is to be increased.

The Dairy-herd-improvement-association program as developed by the extension services in the various States is specifically designed for improving the producing efficiency of the general cow population. The program has been operating in this country for 40 years. Much has been accomplished. In the 40-year period just ending, association herds have been improved until at present the average butterfat production of association cows is 336 pounds per year. The results of the D.H.I.A. program afford sufficient proof that it is possible, practical, and profitable to improve dairy herds.

The butterfat production of the average cow of the Nation is only about 190 pounds per year. The low producing efficiency of the herds as a whole is a challenge to the leaders in the D.H.I.A. program. They must devise methods (1) for more rapidly spreading the improved and successful practices that have been developed in association herds to all herds and (2) for disseminating widely the wealth of information now at hand which will bring about the gradual improvement of all dairy cattle.

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E2. More Accurate Records on More Cows through County Testing Laboratories. J. E. STALLARD, Extension Dairyman, University of Wisconsin.

This paper will show how the number of cows on test has been materially increased in Wisconsin through the county cooperative testing laboratories, even in wartime. It will point out more accurate work because of sufficient and efficient equipment under ideal laboratory conditions. It will show how many herds, not previously interested, have been reached.

It will also show the development of systems and forms that assure greater accuracy in both identification of samples, clear tests, and more efficient use of personnel.

It will show greater ease of supervision from both the state and county levels, with more assistance to Extension by boards of directors.

Illustration by slides and exhibit will be made, if possible.

E3. Evaluation of Pasture in D.H.I.A. Records. R. B. BECKER, University of Florida.

How should pasture be valued in D.H.I.A. records? Should it be on cost of producing the pasture, or on amount of nutrients obtained by the cows? This summary is based on replies from 48 states to 7 points raised in a questionnaire by the Committee on Pastures and Forages?

Methods of valuing pastures frequently vary between associations within a state, as situations and conditions differ. More frequently the D.H.I.A. supervisor sets the charge for pasture, often in conference with the owner, and sometimes the County Agent. The D.H.I.A. members or Boards set pasture prices in 2 states; Extension and Experiment Station staff jointly in 1 state, while the Dairy Specialist advises or furnishes a guide in 4 states. Three grades of pasture, as good, medium, and poor, often are used to indicate quality.

Estimated carrying capacity (amount and quality of forage) is the basis of valuing pastures in 15 states; local rents in 15; some estimate of feed replacement in 7, and cost of producing pasture in 4 states. Generally, little or no distinction is made between permanent and temporary pastures other than amount and quality of forage. Nine states charge rent; 2, cost of production; 3, a higher rate than permanent pastures, and 2, on the basis of feed saved.

Two-thirds of the states reporting distinguished in price between grass, legume, and mixed pastures mainly on the basis of quantity and quality of forage, while one-third indicated no distinction.

"Hay-replacement" values were popular for calculating pasture charges in 3 states growing alfalfa under irrigation. Values were on the basis of hay, less costs of harvest and marketing. One state estimated good pasture equal to one-half the hay and silage replaced. Three states analyze some yearly herd summaries, calculating pasture on a hay basis. In South Carolina, pasture returns are calculated in pounds of "Legume hay equivalent." A "feed level analysis" of yearly records is made in New York, using a pasture yield chart in computations. Feed records in Texas are converted to terms showing the proportion derived from pasture, computed by inverse calculation. In Utah and elsewhere, butterfat production of 138 to 211 pounds per acre of pasture has been computed by inverse calculation. California is making a separate herd management study based on a 1000-lb. cow as an "animal unit," calculating the amount of pasture equivalent to 1,000 pounds of alfalfa hay, or 500 pounds of total digestible nutrients. These methods have been desirable educational tools.

Logical objections to hay-replacement values were expressed regionally, mainly that local hays varied in kind and grade, and that hay prices are established late in the season. The amount of hay or roughage fed to supplement pasture during dry weather is an index to lower pasture charges in several areas.

Winter pastures are used mainly south of the Potomac and Ohio rivers, in the winter wheat belt, and the southwest. Amount of grazing is estimated in 9 states along the northern border of this zone. The supervisor's judgment of benefits derived is the main basis of evaluation in 10 coastal and southwestern states.

A tendency to under-value good pasture was a major criticism. Some definite simple guide is needed to value pastures fairly, either on cost-of-production and maintenance, or a nutritive basis, according to 18 states, several of which are studying the problem Dormancy of some pasture plants in summer drew 3 remarks about annual crops. Economical production from good pastures was emphasized from 3 widely separated areas. Two states are concerned in a "pasture contest" and a "pasture demonstration record." When excellent pastures are charged on a cost-of-maintenance basis, nutrients from pasture are valued under their worth.

It is believed in 1 state that pasture returns can be expressed by the difference between winter feed costs and summer, per 100 pounds of milk. Farmers in 1 state insisted on a flat rate for pasture during the season, regardless of forage yields. None of the suggestions or criticisms mentioned maintaining productivity of the land, although several cost-of-production methods mentioned fertilizer charges.

In general, it appears that pasture valuation should be divided according to regions and practices. More study can be devoted to establishing simple standards for pasture valuation. The question of "cost-of-production" or of "nutrients provided" should be decided by responsible persons in regions, states, or within parts of a state depending on situations peculiar to such areas. The criterion should be how well a method provides the information desired by the cooperating D.H.I.A. members.

Extension Section	*Production Section*
E. T. Itschner, Missouri	R. E. Hodgson, Washington, D. C.
A. R. Merrill, Connecticut	R. H. Lush, Washington, D. C.
E. C. Scheidenhelm, New Jersey	R. B. Becker, Florida, Chairman

E5. Effective Use of Herd Records. RUSSELL E. HORWOOD, Michigan State College, East Lansing, Michigan.

Dairy farming is a business. In many sections and on many farms it is the major agricultural enterprise. Record keeping is an important part of any business. It is an essential chore the same as feeding, milking or testing. The extent of the value of records depends upon their use in developing and maintaining an efficient herd. The following charts indicate ways of summarizing records to give a clearer picture of the exact situation within a herd. A study of the conditions indicated by the charts will suggest procedures that will aid in placing the herd on a more efficient basis. Additional studies of a similar nature may be made following the plan outlined. *The greatest value in keeping such records may come from comparing the current record* with similar records kept in previous years.

Chart 1. If this chart is completed each year it will provide a convenient summary of the number of animals in the herd by age groups. It will provide much of the information upon which a two-year sales program for females and a year's program for bulls may be based. In addition the data should be an aid in developing an inventory of the herd.

NUMBER OF ANIMALS IN HERD AT END OF TESTING YEAR

Year	Calves under 6 mos.		Older bulls for sale	Heifers 6 mos. to freshening	Cows
	Heifers	Bulls			

Chart 2. When this chart is completed it may show that you have a young herd. In such a case a larger sales program may be planned than if the average age of the milking herd is further advanced. It may be an aid in deciding upon the animals to sell if these data are compared with the production data in Charts 5 and 6. It is well to keep in mind that on the average a cow stays in the herd only four years. If the herd can be operated

AGE IN YEARS OF COWS IN HERD AT END OF TESTING YEAR[1]
(Designate cows by name or herd number)

Year	Range in age (years)										
	2	3	4	5	6	7	8	9	10	11	Over 12

to extend the useful life of the animals beyond this period it increases efficiency greatly.

Chart 3. A study of the data in this chart will indicate the success the herd is having in breeding heifers to freshen at the desired time and age. If results are not satisfactory it will be important to determine the cause. It may be due to carelessness, lack of fertility on the part of the bull, or disease in the heifers. When the cause is determined steps may be taken to change the condition. Heifers freshening at an advanced age may generally be expected to produce more during the first lactation. Likewise the opposite may be expected from heifers that freshen at a very young age. Records made under such conditions frequently lead to over or under evaluation of such animals from the standpoint of future production and production expectancy in their offspring.

AGE IN MONTHS OF HEIFERS AT FRESHENING DURING TESTING YEAR[1]
(Designate heifers by name of herd number)

Year	Range in age (months)										
	24	25	26	27	28	29	30	31	32	33	Over 34

[1] Compute average age at end of each year.

Chart 4. The data in this chart will reveal the freshening interval for each cow and for the herd as a whole. This information has been given little consideration except when the interval has been unduly long. In general, dairymen prefer to have cows freshen at 12-month intervals. Studies made in Iowa herds carrying on Dairy Herd Improvement Association testing show the average interval is approximately 14 months. A study made in Michigan shows a higher average daily fat production for cows with a freshening interval less than 365 days as compared to longer freshening intervals. The shorter freshening interval will tend to increase the herd average. It will also lower the individual lactation record. The lower lactation records may reduce somewhat the prices that cattle may be sold for until more consideration is given on the part of the purchaser to the length of the record and the freshening interval in connection with the record. There is a trend toward greater use of the 10-month record.

FRESHENING INTERVAL IN MONTHS OF COWS IN HERD[2]
(Designate cows by name or herd number)

Year	Range in months								
	11	12	13	14	15	16	17	18	Over 18

[2] Compute average interval at end of each year.

The interval may generally be shortened by breeding cows at the first heat period following approximately six weeks after freshening. If the cow does not conceive on this breeding there is an opportunity to breed again at the next period and maintain a 12-month interval. The number of services per conception is close to two on the average. It is generally believed that cows bred in the earlier heat periods following freshening conceive more readily.

Chart 5. The high lactation records of the cows in your herd as listed here may point to the individual animals that have the greatest producing ability. Abnormal factors such as a long freshening interval, cystic ovaries and excessive feeding should be considered to avoid over evaluating such an animal. Likewise, the low production of some cows should not lead to underevaluation if interfered with by such factors as mastitis, abortions, or underfeeding. The repeatability of performance should be watched in each animal and also in her offspring.

Distribution of High Individual Records of Cows in Herd at End of Testing Year Regardless When Made
(Designate cows by name or herd number)

Year	Range in production (lbs. fat)							
	Heifers incomplete	200–300	300–400	400–500	500–600	600–700	Over 700	Average

Chart 6. A study of the distribution of lactation records within the herd each year will indicate the range of production that exists. The high records will indicate to some extent the inheritance for production that the sires to be used should carry. It will point out to some extent the lower producing animals that may be profitably eliminated. The repeatability of records may be observed if the data are studied from year to year.

Distribution of Lactation Records Completed During Year
(Designate cows by name or herd number

Year	Range in production (lbs. fat)							
	Heifers incomplete	200–300	300–400	400–500	500–600	600–700	Over 700	Average production

Chart 7. The loss of cows from the herd from causes other than dairy purposes in various studies runs from 15 to 20%. An actual record of such losses within a herd from year to year will indicate the place to put the major stress to overcome this. If it is due to low production it may be

caused by several factors as poor bulls, poor feeding or management, or a combination. Excessive loss due to mastitis would indicate an improved mastitis control program. Shy breeders may indicate greater use of a good veterinarian.

CAUSE OF REMOVAL OF COWS
(Designate cows by name or herd number)

Year	Number of cows in herd	Dairy purposes	Low production	Removal mastitis	Shy breeders	Other causes	Per cent of cows lost

Chart 8. Studies in several states indicate that from 12 to 20 per cent of the female calves born never become producing cows. This is a tremendous loss. The extent of these losses and their cause is important to the herd operator. After they are known a program to overcome them may be of utmost value. The calves must be raised for herd replacement, or sale if the full value of the inheritance of the sire and the cow are to be secured. Greater returns may be expected if these losses are kept at a minimum.

DISPOSITION OF CALVES BORN

Years	Sex	Number born	Intentions for		Sold for		Died	Age	Cause	Per cent loss
			Replacement	Sale	Breeding	Slaughter				
	Male									
	Female									

BREEDS RELATIONS COMMITTEE REPORT

B. Program of the Purebred Dairy Cattle Association. G. A. BOWLING, Port Chester, New York.

The objectives of the Purebred Dairy Cattle Association are set forth and a brief summary is given of the following projects, which are a part of the program of the Association.

1. Uniform Score Cards for Dairy Cattle.
2. Uniform Rules of Official Testing.
3. Uniform Rules for Proving Sires and Dams.
4. Uniform Rules for Artificial Insemination.
5. Uniform Rules for Herd Type Classification.
6. Rules and Ethics for Public and Private Sales.
7. Uniform Rules for Interstate Movement of Dairy Cattle.
8. Uniform Classes for Dairy Cattle at County and State Fairs.
9. Better facilities for Livestock Photography at State and National shows.
10. A Dairy Cattle Breeding Research Council.

The relationship of the Association to the breed registry organizations and the general dairy cattle improvement program is explained.

D. Uniform Rules for Official Testing. Floyd Johnston, Iowa State College, Ames, Iowa.

Since April, 1941, a joint committee representing the American Dairy Science Association and the Purebred Dairy Cattle Association has been working on uniform rules for official tests which can be used by all dairy breed registry organizations.

These rules amalgamate those adopted by the American Dairy Science Association with those recommended by the Purebred Dairy Cattle Association and show the points of difference, where there are any, from the uniform rules of each of the five breed registry organizations.

When put into use, the one set of regulations will replace the 7 now in use and will be a great forward step toward uniformity and the results will be most helpful to the colleges and breeders and to students of the official testing program.

F. Program Suggestions for Inter-Breed Groups. E. C. Scheidenhelm, Rutgers University.

Programs developed by inter-breed groups within states should, in a large measure, develop an action program on a state level similar to that of the national breed associations on a national basis.

The success of state groups depends upon the leadership they have and the desire of the breed groups to "give and take" to help reach a reasonable solution to this problem.

Activities can largely be grouped into two classes, namely; educational and non-educational.

I. Educational.
  a) Active support to the 4-H club program on a uniform basis.
  b) Uniform program in recognizing meritorious sires.
  c) Assistance in arranging programs for Farmers' Week programs.
  d) Suggestions for extension program on breeding, programs, production testing, etc.
  e) Assistance to artificial breeding groups in setting up standards for sires and otherwise aiding the breeds with mutual problems.
  f) Educational dairy programs at fairs and exhibitions within states.
  g) Recognition of outstanding leadership to the industry.

II. Non-educational.
  1. Securing proper legislation for health regulations.
  2. Developing uniform standards for dairy shows on county, district and state bases.

3. Setting up ethics for sales.
4. Developing an advertising program for cattle industry of the State.

The success of an inter-breed organization depends upon the continuity of activity. At least three or four meetings should be held during a year. Representatives to the inter-breed group from the respective participating breed organizations should always keep their own groups fully informed of the activities of all meetings.

ARTIFICIAL BREEDING

E9. Some New Management Methods for Artificial Breeding Associations. E. J. PERRY, Rutgers University.

Among some of the newer methods of management reported as now being practiced by artificial breeding units in a number of the states and one foreign country are the following: 1) The use of a standard letter and a well-prepared set of questions and answers for mailing to prospective members, 2) A state association council of breeding associations to aid in unifying and standardizing the program and helping to determine policies of state-wide interest, 3) A high minimum standard of genetic excellence for both proved and young bulls, which must be met before they are acceptable for cooperative breeding project (utilizing the breed average or other measure), 4) The setting up of bull-testing stations—a special plan for improved control of environment in proving bulls by assembling sufficient daughters two months prior to freshening and subjecting them to identical feeding and management methods while on production test, 5) Giving additional free services, above the first three to "trouble cows" when the whole herd is enrolled, 6) Having a state-wide standard method of clean-up for technicians before leaving the farm, 7) Advertising in local papers using a key headline and presenting new important current information each week or month dealing with the bulls' progeny, production and type, etc., 8) A central clearing house on sire information to which reports on all bulls purchased, leased or rejected will be sent, 9) Special on responsibility to members in new territory, making certain that they get accurate information regarding results to be expected, thus preventing a good many disappointments, 10) Buying or leasing bulls on fertility contracts, 11) Supplying all members with cow breeding and management calendars, 12) To reduce chance of syringe contamination to a minimum either refrigerate syringe after each insemination or use dry syringe along with an inseminating rod that is large enough to carry a little more than a cc. of semen.

A number of these practices are meeting with favor because of results produced and are therefore, deserving of careful consideration by associations that have not yet adopted them.

E10. Schools and Program for Training Inseminators. Raymond Albrectson, Cornell University.

It is self-evident that the success of an artificial breeding association rests heavily on the skill, tact and efficiency of the inseminator employed. Good organization, large cow enrollment and membership enthusiasm rapidly deteriorate under the corroding effect of poor conception rates attributable to poor technique. Basically, farmers' cows must be gotten with calf artificially at an efficiency closely approximating or equal to natural service. In addition to good insemination technique, a technician must have a well-developed sense of responsibility, a pleasing personality and an enthusiastic appreciation and understanding of the fundamental purpose of the program, viz., herd improvement through use of desirably proved sires.

It became apparent early in the development of this program in New York that a definite system of training schools or short courses for insemination technicians was necessary. Since 1941, twenty-five short courses have been held with a total enrollment of 474. Definite scholastic requirements are not prerequisite. A history of dairy farm experience is considered desirable. Experience has shown the men under 21 years of age and those lacking a farm background to be disappointing in the course and out in the field. No other objective measures have been found to date that are reasonably reliable in selecting enrollees.

The organization of the short course is such as to give as much usable basic information and practice as possible in eleven days. All presentations are designed to stimulate each enrollee's ability and aptitudes. That is all any short course can hope to do. The subjects are arranged in a sequence very similar to the development of the program in the field. Lectures on physiology, bacteriology, genetics, organization and record keeping are supplemented with practice periods on insemination technique, preparation and sterilization of equipment and financial and breeding records. Emphasis is placed on correct procedures. Each enrollee has his work checked as to its quality in every operation. About one-half of the entire course is given over to insemination practice and closely allied techniques.

A short course of this kind cannot produce *completely trained technicians*. It is impossible to do that in the short time allotted. Experience has shown conclusively that men attaining a high rating in the course have become good technicians under field conditions. Equally conclusive is the unsatisfactory performance of men rated in the lower half of the class. It appears that the aptitude for this work is an attribute not possessed by all people.

A system of written quizzes and careful observation of practice techniques is used to grade the enrollees. The personality, attitude and previous experience and training of the enrollee is also considerd in the final grading. The highest grade given is "1"; the lowest is "5." Enrollees earning a grade of "1" or "2" will invariably be successful. Some grade "3" men will not be successful. Men earning grades below "3" cannot become approved technicians in New York.

After employment each technician is checked by either an Extension Specialist or an inseminator-at-large employed by the New York Artificial Breeders' Cooperative, Inc. This check-up is for the purpose of giving the new technician confidence and to forestall undesirable habits or practices.

The inseminator training course as given in New York has gone through many progressive steps in its development. There appears to be several improvements that can be made to strengthen the effectiveness of this short course. Among them are the following:

1. A better means of selecting candidates.
2. Limitation of total enrollment to a maximum of 40.
3. Improve teaching methods—simplified instructions plus visual aids.
4. More practice periods, possibly fewer lectures.

4-H CLUB WORK, QUALITY PROGRAMS

E11. New Developments in Calf Club Work in Pennsylvania. J. C. NAGEOTTE, Pennsylvania State College.

Our calf club work in Pennsylvania has suffered in quality during the war years. We have had fewer meetings, shortage in labor has caused a deterioration in the quality of the work, good calves have been higher priced and fewer can be obtained and restrictions in travel have practically cut out calf club shows. The added responsibilities put on the county agents has also cut down on the attention the club members received. It has not been easy to correct this. We feel that our biggest loss has been in the showing program, and that if this is corrected the other things will automatically fall in line.

As fast as we can get it adopted we are starting a program of calf club round-ups in the communities. At these round-ups the club members are scored on the quality of the calf selected, on their showmanship, on the way the calf is developed and fitted for the show, and on their calf club record book. The best calves are selected at these community round-ups to be shown at a county show where they will be only judged on type with extra prizes awarded for the best fitted animals and the best shown animals. At these county shows a limited number of animals from each breed are selected to represent the county in a Regional 4-H Show where the animals will be judged on type as individuals within the breeds and in county groups of four or five for trophies. In no case will cash awards be given for placing, only ribbons are awarded for placing and what money the club members receive is given as a transportation allowance and each receive the same allowance for the distance traveled. The county furnishes their own money for the county shows and we have a promise from the State Breed Associations to support the Regional 4–H Shows on this basis. In the case where grade calves are owned they are only shown in the community round-ups. All the calves shown in the county and regional shows are registered calves and transferred to the club members.

A new development has entered into the picture this year with the American Jersey Cattle Club sponsoring a Junior Show at Columbus, Ohio, the second week in October. We were belatedly put on the advisory committee for this show and had no chance to work on the rules until they were set up and adopted. It is our thought that we can select our Jersey calves for the National Show at these Regional Shows and in this way make a calf club member and his calf earn their right to work up to the peak in the National Show. We recognize that this National Show will possibly have the same faults that the National Dairy Show had when the calf club calves were exhibited; that is the exploitation of club members for the benefit of some large breeders and breed associations. However, I feel that we can do more good on the inside working with them than we can by being on the outside criticizing what other people do when we don't participate. If each state will see to it that the exhibit they send to this show will be strictly a 4–H dairy exhibit in all sense of the word there cannot be much exploitation. If we all participate with the right spirit this commendable project could be carried on by all the breed associations cooperating and it would be a real incentive for club members to strive to get the best calves and do the best job in developing them, and finally in developing good herds for themselves.

E12. Extension Work in Quality Improvement of Dairy Products.

AMER B. NYSTROM, Dairy Husbandman, Bureau of Dairy Industry, U. S. Department of Agriculture.

The paper emphasizes three specific procedures as follows:

1. See that in each State the whole college dairy staff—including instructors, research workers, and extension specialists—is thoroughly sold on the idea of improving the quality of milk and cream and that each member of the staff has a definite part in working out the plan for the State.
2. See that the plan includes a definite program for teaching rural youth the principles and practices of clean milk production, especially through 4–H dairy club and vocational agricultural activities.
3. Make full use of the processors and the managers of receiving stations in the quality improvement program by cooperating fully with them, both directly and through the State departments of agriculture and the State departments of health.

The address of Prof. R. B. Stoltz on "Organize for Quality," given before the American Butter Institute's annual convention in November, 1945, was commended for reading. The following quotation from his address was cited:

"... any program to improve quality must be done by the creamery men themselves and not by the law enforcement officer nor the extension man in the College of Agriculture, nor the representative of the State Department of Agriculture. They can cooperate and work with them but the job must be done by the creamery men themselves. It is not necessary to have 100% participation, but you do have to have a majority, and probably 75 or 80%, and, of course, the greater the percentage, the easier the job is accomplished. I want to convince you creamery men that it is your responsibility, that you are the center of the universe when it comes to the quality, that you can't "pass the buck" to the law enforcement officer, your competitor or someone else. It rests upon your shoulders, for what you do will determine what the industry accomplishes."

MANUFACTURING SECTION

DRY MILK PRODUCTS

M1. A New Quantitative Method for Determining the Solubility of Milk Powders.

J. FRANK CONE AND U. S. ASHWORTH, Department of Dairy Husbandry, State College of Washington, Pullman, Washington.

In a search for a more sensitive test than the American Dry Milk Institute (ADMI) method for determining the solubility of milk powders, we investigated less drastic methods for the reconstitution of the powder and also means of making the test more strictly quantitative. We have developed a procedure that differentiates quite reliably between powders that by the ADMI method appear to be almost completely soluble. It differs from the ADMI method in three main features: (1) Reconstitution is accomplished by a standardized procedure of manually shaking the powder and water in a flask instead of the vigorous mechanical agitation used in the ADMI method. (2) The entire reconstituted sample is employed instead of only a portion, as in the ADMI method. (3) The centrifuged sediment is not measured in volume units but is dried and weighed so that the results can be calculated in terms of per cent solubility.

Samples of spray-dried powder tested by the new method differed markedly in their solubilities, but for each sample the results were reproducible within narrow limits.

M2. Drying Milk by the Use of Cold.

O. F. HUNZIKER, La Grange, Illinois.

Processes developed for drying organic substances by the use of cold have to do with drying from the frozen state. The prior art on this subject reveals U. S. patents granted as early as the beginning of the present century. These processes are essentially intended for batch operation. While they undoubtedly can be worked and a dry product can be produced, they are not considered of really practical importance and have not reached the stage of commercial development.

The basic principle involved in this method of drying is to subject the product to a very high vacuum. For example, if whole milk is subjected to a very low absolute pressure (high vacuum) such as 1/50-inch absolute pressure, the water is frozen out of the product in the form of very small ice crystals and the solid material that is left is practically bone dry. If the high vacuum is maintained and heat is applied to the product the ice will sublime, *i.e.*, it will change from a solid state (ice) to a gas (water vapor) without going through the liquefying state (water).

The success of the process depends upon the high vacuum and its sustenance. But the water vapor created during evaporation is very elastic material. As the pressure decreases (vacuum increases) the specific volume of water vapor increases. At atmospheric pressure at the sea level, 1 lb. of water vapor occupies approximately 13 cu. ft., but under high vacuum (absolute pressure 1/50 inch) each pound of water driven off from the product by sublimation of the ice crystals in the form of water vapor, occupies approximately 30,000 cu. ft. Hence this tremendous volume of water vapor must be removed or dissipated at the same rate in which it is being formed, otherwise it would be impossible to maintain the very high vacuum that is essential for the success of the process.

Within the past few years this and related engineering problems that have blocked the successful application of drying milk in the frozen state, have yielded to the experimental studies of the research staff of the Chain Belt Company. This has resulted in the development of the Freeze Drying process. In this process so high a vacuum is maintained in the drying chamber that dehydration is accomplished in an atmosphere essentially devoid of atmospheric air. The process yields a powder of exceptionally low moisture content and the oxygen content of the powder is sufficiently low to eliminate the necessity of vacuumizing the filled can. The process thus promises maximum resistance to fat oxidation and prolonged shelf life of dried whole milk and other dried milk products with relatively high butter fat content.

M3. Biochemical Changes in Whole Milk and Ice Cream Mix Powders During Storage.[1] N. P. TARÀSSUK AND E. L. JACK, Division of Dairy Industry, University of California, Davis, California.

The biochemical changes occurring in dry milk products during storage affect the palatability, and thus the market quality, of these products. These changes are influenced by the processing treatments, the storage temperature, and the oxygen level in the package. Studies here presented show some of the effects of different conditions on the changes occurring in powders:

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[1] The subject matter of this paper has been undertaken in cooperation with the Quartermaster Corps Committee on Food Research.

1. *Processing treatments.* Preheating temperatures are of major importance in retarding the deterioration of powders by staling. Powders made from milk preheated to 190–195° F. were consistently superior to powders made from milk preheated only to 165° F. This was particularly evident at room-temperature storage, whereas deterioration at 100° F. was sufficiently rapid to mask the effects of preheating. The effect of preheating was on the material associated with the fat because when the milk was separated and the skim portion preheated to 190–195° F. and the cream to lower temperatures no improvement was noted, while powder made from skim milk had excellent keeping quality whether preheated to either temperature.

2. *Storage temperature.* Observations were made at room temperature and 100° F. Atmospheric-packed samples had poor keeping quality at either temperature. When packed under low oxygen level—below two per cent—whole milk powder and ice cream mix powder were still acceptable after eleven months storage at either temperature. Slight staleness was noted. The rate of deterioration was greatest in the first three months of storage.

3. *Oxygen level.* Oxygen is the chief contributing factor to the development of stale flavor. Samples of whole milk powder packed with oxygen-absorbents, pyragallol and chromous chloride, to maintain the oxygen level as low as possible showed no deterioration in flavor at seven months. There was also no change in the composition of the headspace gas showing that no reactions occurred in the powders.

The source of stale flavor seems to be in the material associated with the fat because powder made from skim milk, whether packed in atmosphere or nitrogen, did not develop stale flavor, whereas powder made from buttermilk developed typical stale flavor.

The quantitative measurement of the changes accompanying deterioration of powders furnishes some ending as to the mechanism of the reactions involved. Analyses of the head-space gas show that production of carbon dioxide is a feature of deterioration. This gas is one of the final products of these changes. Amounts of carbon dioxide up to four per cent were produced in atmosphere-packed samples at 100° F., but at room temperature only up to 0.5 per cent, while samples packed with oxygen absorbents evolved practically no carbon dioxide. These may not represent absolute amounts of carbon dioxide because of the absorbing capacity of milk powders for this gas.

The reducing activity of powders increases in 100° F. storage, but is slight or non-existent at room temperature. Samples packed in nitrogen showed greater reducing activity than air-packed samples. There was no relationship apparent between the reducing activity and the organoleptic score of the powder.

M4. The Role of Moisture and Oxygen Levels in the Changes Produced in Dry Ice Cream Mix in Storage. R. W. KUNKEL, S. T. COULTER, AND W. B. COMBS, Dairy Division, University of Minnesota.

Dry ice cream mix may undergo a number of changes in storage, including the development of tallowy and stale flavors, browning, production of carbon dioxide and water, utilization of oxygen, increase in reducing substances, in fluorescing materials (acetone, ether and 10 per cent potassium chloride extracts), in acidity and a decrease in solubility. Noticeably tallowy flavors do not develop in dry ice cream mix at oxygen levels below the Quartermaster Corps, Army Service Forces specification maximum of 3 per cent; however, stale flavors may develop even at oxygen levels as low as 0.5 per cent.

Samples of dry ice cream mix containing 1 to 3 per cent moisture, gas-packed at equilibrium oxygen levels of 0.5 to 3 per cent were stored at 100° F. (38° C.). The rate of flavor deterioration was accelerated by increases both in moisture and oxygen levels, but the effect of the moisture level was much the greater. Samples of dry ice cream mix ranging in moisture content from 2 to 4.5 per cent and gas-packed at equilibrium oxygen concentrations varying from 0.3 to 40 per cent were stored at 140° F. (60° C.). Under these conditions, the rates of increase in fluorescence, reducing substances, carbon dioxide, browning, and moisture, the decrease in pH and the loss of solubility, all were greatly accelerated by increase in the moisture level but were influenced very slightly—if at all—by changes in the oxygen level. Data at hand are not entirely adequate to establish the maximum moisture level desirable in ice cream mix, but this figure is not above 1 per cent.

M5. Factors Related to the Brown Discoloration of Powdered Whole Milk. W. A. KRIENKE AND P. H. TRACY, University of Illinois.

It has been observed that when dried whole milk is stored in steel barrels a few hours prior to packaging, as is done by some commercial companies, that a brown discoloration may take place if the powder is not cooled as it leaves the drier. To gain more information regarding this defect this study was made.

The experimental samples of powder were heated in Pyrex test tubes (1.5 x 15 cm.) in a temperature-controlled oil bath. Temperatures varying from 70° to 140° C. were used. The tubes were either left open, stoppered (with pressure relief tube) or were vacuated. Different moisture levels were obtained by exposing the powder to the atmosphere and to vacuation previous to heating. Gassed and ungassed samples were used for studying the significance of the oxygen content.

The data obtained show that discoloration of dried milk is related to: (1) The temperature and time of exposure of the powder. Discoloration was found to take place at all temperatures studied. In no case was it found possible to obtain the extent of discoloration in the test tube samples that was observed in the barreled powder when temperatures comparable to that of the powder as it reached the storage barrels were used. (2) Discoloration took place to a greater degree in the powder held in stoppered containers. The least discoloration took place in the samples vacuated during heating. (3) As the moisture content of the powder was decreased, the extent of browning became less. The effect of moisture was found to be very significant. (4) Differences in the discoloration of samples with varying oxygen levels were no more than would be expected from differences in moisture content as brought about by the vacuation.

Evidence was also obtained showing that both moisture and carbon dioxide were given off as a result of the browning reaction.

M6. Relative Keeping Qualities of Frozen Milk, Frozen Concentrated Milk, and Spray-Dried Whole Milk. J. H. HETRICK, W. A. KRIENKE, AND P. H. TRACY, University of Illinois.

Studies have been conducted on the effects of ratio of concentration, addition of dextrose before freezing and method of freezing on the keeping quality of the products stored in a frozen state – 12 to – 15° F. in paper containers. Milk powders manufactured from the same lots of milk concentrated approximately 3–1 were air-packed and nitrogen-packed in tin containers and stored both at room temperature and at – 12° F. to – 15° F. Samples of each were analyzed initially for per cent of fat, per cent of total solids, acidity, reduced ascorbic acid, bacteria count, flavor and copper and iron content. At intervals during the storage period thawing observations, ascorbic acid and flavor scores were made on the thawed products; analyses of the headspace gas of nitrogen-packed powder were made and ascorbic acid and flavor scores were determined on the reconstituted powdered milk both air-packed and nitrogen-packed. In one experiment, sixteen periodic observations were made over a storage period of 523 days. A series of samples made from another lot of milk was stored 365 days and twelve periodic observations were made. A third experiment involving only frozen milk, frozen milk concentrated approximately 2–1, and frozen milk concentrated approximately 3–1 was performed in which observations were made throughout a 233 days storage period.

In general the frozen milk, and frozen concentrated milk possessed better keeping quality than even gas-packed whole milk powder made from the same lot of milk and stored at the same temperature. The flavor of the frozen products remained satisfactory throughout the 523-day storage period, while the gas-packed whole milk powder stored at the same temperature became unsatisfactory after a storage period of 338 days. The air-pack powder stored at – 12° F. possessed a metallic flavor after 175 days. When powder was stored at room temperature an oxidized flavor was observed at 198 days in the nitrogen pack and at 105 days the air pack became unsatisfactory. There was some difference in the copper and iron content among these samples but the highest copper content did not exceed 1.5 micrograms copper per gram of solids.

All samples of frozen milk and frozen condensed milk showed no curdy, oily or flaky appearance on thawing when stored for 365 days at – 12 to – 15° F. One sample of milk concentrated 3–1 was satisfactory even after 523 days storage.

Ascorbic acid values on the thawed frozen products varied considerably but in general the retention of ascorbic acid in the frozen products compared favorably to that in gas-packed milk powder stored at the same temperature.

In the milk powder samples, greater ascorbic acid loss occurred in the air packs than nitrogen packs and more loss of ascorbic acid occurred in those stored at room temperature than in those stored at – 12° F.

Greater amounts of oxygen were present in the head space of gas-packed milk powders stored at – 12° F. than at room temperature.

The ratio of concentration (0 to approximately 3½–1), addition of dextrose prior to freezing or drying, the method of freezing (placing at – 12° F. vs. freezing initially in continuous ice cream freezer) did not prove to be important factors in flavor changes, or ascorbic acid retention, on thawing of the frozen products.

M7. Physico-Chemical Factors Affecting the Reconstitutability of Dry Whole Milk. G. H. WILSTER, O. M. SCHREITER, AND P. H. TRACY, University of Illinois.

The problem has involved three phases: (1) Influence of manufacturing methods as affecting the reconstitutability, (2) method used for reconstituting, and (3) causes of film and scum deposit on containers used for reconstituted milk.

In these studies milk of highest standard consumer's grade and highest standard manufacturing grade was used. The equipment available for manufacturing the dry milk included: 200-gal. capacity stainless steel spray pasteurizer, 3-ft. diameter stainless steel vacuum pan, 200-gal.-per-hour two-stage homogenizer, 10-gal. capacity pre-heating tanks, 75-gal.-per-hour pressure pump for spraying, 6′ × 17′ × 9′ drying chamber, and the necessary equipment for can sealing, evacuation and gassing of cans.

Typical defects of the reconstituted milk were: (1) Presence of undispersed powder particles which in some instances could be visibly observed adhering to the wall of the container, (2) scum on surface of milk, (3) formation of distinct layers at top and bottom of container when this was left undisturbed for several hours in a refrigerator.

The film deposit on the container wall sometimes contained more and sometimes less fat in proportion to the total solids than the reconstituted milk.

Of the factors studied so far that have favorably influenced the reconstitutability were: (1) small powder particles and (2) the addition of a small amount of sodium citrate as a protein stabilizer to the condensed milk before spraying. The film deposit and scum have been reduced by: (1) manufacturing small particle-size powder, (2) spraying homogenized condensed milk containing minute, uniform-sized fat globules, (3) not overheating the powder in the drying chamber.

The factors that have favorably influenced the completeness of the dispersion of the dry milk particles in the water used for reconstituting were: (1) Using warm, 110° F., water instead of water at 75° F. or lower, (2) heating the dry milk–water mixture to a temperature of 150° F., (3) homogenization of the reconstituted milk at a temperature of 75° F. and a pressure of 1,000 to 2,000 pounds per square inch, (4) passing the reconstituted milk through a clarifier, (5) using a high-speed electric agitator when reconstituting, and (6) the addition of a small amount of sodium citrate to the water used for reconstituting.

The results have shown that, to produce reconstituted dry whole milk which compares favorably with fresh, homogenized milk in physical condition, when water at 75° F. and an electric malted milk mixer are used when reconstituting, the condensed milk sprayed must contain the fat globules in a finely dispersed form, the powder particles must be uniformly small and overheating during drying must be avoided.

M8. Changes in the Fluorescence of Dry Whole Milk on Storage.

R. JENNESS, S. T. COULTER, AND R. LARSON, University of Minnesota.

The production of fluorescing and brown-colored substances in dried milk during storage has been studied because such substances appear to be formed under conditions that favor stale flavor deteriorations, and it was hoped that the intensity of fluorescence or of brown color might serve as an objective index of the extent of stale flavor development.

A method has been devised whereby the fluorescing materials are divided among three successive extracts of the reconstituted dry milk as follows: I—acetone-water (67 : 33) ; II—acetone-ether (20: 80) ; and III—10% KCl. Fluorescence of each extract was determined with the Coleman Photofluorometer using filters B–1 and PC–1, the instrument being standardized with a quinine sulfate solution. Riboflavin can be determined independently in extract I using filters B–2 and PC–2.

Upon storage of dry whole milk the fluorescence of extracts I and III increased while that of extract II was not consistently altered. The development of fluorescence (and of brown color) can be greatly accelerated by increasing the moisture content of the powder or the temperature at which it is aged. Furthermore, there is a certain degree of parallelism between the rates of development of stale flavor and of fluorescence. The oxygen level during storage appears not to be related to the development of fluorescing and brown materials.

The analytical scheme outlined has been further applied to several series of simplified systems of systematically varied composition in order to ascertain which of the milk constituents are responsible for (1) the initial fluorescence and (2) the fluorescence developed in storage. It appears in fresh samples that riboflavin is responsible for about 50% of the fluorescence of extract I, the remainder being extracted from the various protein constituents. The lipides account for most of the fluorescence of extract II while the proteins contribute to that of extract III.

Upon storage the increases in fluorescence of extract I appear to involve the proteins, lactose and riboflavin, while those in extract III are associated with the proteins and lactose.

M9. Changes in Reducing Materials in Dry Whole Milk During Storage.

L. K. Crowe, S. T. Coulter, and R. Jenness, University of Minnesota.

The effect of storage of dry whole milk at 60° C. on the production of materials which reduce 2,6-dichloro-phenol-indophenol and potassium ferricyanide under specific conditions has been studied.

Materials which reduce ferricyanide increase during storage.

Moisture is important in the reaction which produces materials which reduce ferricyanide. The rate of the reaction in low-moisture powders does not appear to be greatly influenced by the oxygen level.

Reduction of indophenol by materials in the protein-free filtrates from whole milk powders indicates an apparent destruction of ascorbic acid in air-packed powders at all moisture levels studied.

The slight increase in indophenol titre of samples stored at 60° C. for periods of time up to 192 hours may probably be attributed to the production of indophenol-reducing materials which remain in the protein-free filtrate.

M10. Drying Milk in an Atmosphere of Nitrogen or Carbon Dioxide.

S. T. Coulter, Dairy Division, University of Minnesota.

The use of a gas such as nitrogen or carbon dioxide in spray-drying has been demonstrated to be feasible. Dehumidification of the gas for recirculation can be accomplished by cooling to 70° F. Cooling can be effected by a suitable water coil using about 50 gallons per minute of 50° F. water per 1000 C.F.M. of nitrogen. Obviously the spray-drying system must be reasonably gas-tight.

The principal advantage of drying in an atmosphere of an inert gas is the ease with which extremely low oxygen levels in the canned powder can be reached. Based on the results of numerous trials, air-dried powder gas-packed to an equivalent oxygen level has essentially the same keeping quality as the product dried in a gas of low oxygen content. With the experimental drier at the University of Minnesota, it has been possible to dry and can milk in gas containing less than 0.5 per cent oxygen. To gas-pack air-dried powder to this oxygen level requires triple or quadruple gassing.

M11. Relation of the Temperature of Separation and the Heat Treatment Given the Skim Milk to the Keeping Quality of Spray-Dried Ice Cream Mix.

HARRY PYENSON AND P. H. TRACY, Department of Dairy Husbandry, University of Illinois, Urbana, Illinois.

Twelve batches of dried ice cream mix were made from the same lot of milk. The milk was separated at 55° and 130° F. The skim milk was divided into two lots and forewarmed at 150° F. for 20 minutes and 180° F. for 5 minutes, and condensed in a stainless steel vacuum pan. Some of the cream was made into butter and some was made into butter oil. Dried ice cream mixes were made from these ingredients so that the relation of the heat treatment given the serum solids to the keeping quality could be studied.

The carbon dioxide content of the headspace gas varied throughout the storage period. Oxygen concentration gradually became lower as the storage period advanced. There was a tendency toward more rapid oxygen absorption, more carbon dioxide evolved, and more oxidized flavor development in mixes that had the condensed skim milk forewarmed at 150° F. for 20 minutes than in those mixes that had the condensed skim milk forewarmed at 180° F. for 5 minutes. There seemed to be no significant difference in oxidized flavor development when the mixes were prepared from concentrated skim milk made from skim milk separated at 55° or 130° F. Whether the fat used was in the form of cream, butter or butter oil was not a factor influencing the keeping quality of the dried ice cream mix. All the batches became oxidized which contained butter oil, butter or cream which were made with skim milk forewarmed at 150° F. for 20 minutes. After one year dried mixes made from skim milk forewarmed at 180° F. for 5 minutes had not developed an oxidized flavor and were still highly palatable.

High forewarming temperature on the skim milk before condensing is beneficial in prolonging the keeping qualities of dried ice cream mixes and the retardation of oxidized flavor development.

SYMPOSIUM

Summary of Quality Symposium. H. F. JUDKINS, *Chairman.* Panel Members: G. M. TROUT, C. V. CHRISTIANSEN, A. C. DAHLBERG, E. C. SCOTT, R. V. HUSSONG, G. W. SHADWICK, W. V. PRICE, H. L. WILSON, P. H. TRACY, O. F. GARRETT.

In Mr. Judkins' remarks opening the Symposium, he pointed out that in this seller's market era, interest in quality was inclined to lag and that a Quality Symposium, therefore, seemed very timely. It was stated that certain minimum facilities must be available and that these facilities must be properly used by quality-minded individuals if a quality product is to be maintained. Emphasis was placed on the importance of university instruction in teaching students the best minimum facilities for various dairy operations and the art of teaching proper methods in the use of these facilities. It was suggested that a course might well be given covering product defects, their causes and prevention, in order to train students in the fundamentals of "diagnosing quality disease."

Examples were given relating to the effect of improper equipment or lack of equipment to quality.

It was pointed out that only high quality raw materials can be used, shortage or no shortage.

In conclusion, Mr. Judkins stated that if management wants quality and is as interested in studying quality reports as they are profit and loss statements, they will have quality.

The different members of the panel had been asked to submit a series of questions and answers in advance to Mr. Judkins. Prior to the Symposium, these had been arranged in the form of a radio script so that the Chairman could ask related questions about the quality of the different products and these were answered by members of the panel. These questions and answers were interspersed with frequent questions and discussion from the audience. Space will not permit a detailed report on the questions and answers. The following are some of the conclusions which were developed:

1. Standards for quality are mainly determined by the results of research on the part of those who are students of quality. Regulatory bodies set their standards based on such research.
2. The consumer's part in determining product quality is principally in judging whether or not the standards established by the expert suit their senses of sight and taste. This consumer judgment is reflected principally in the sales curve for the product and to a much lesser degree in quality complaints.
3. Quality complaints are relatively few and this fact is a deterrent to progress in quality improvement.
4. The quality control supervisor has two main functions; first, to maintain uniformity of quality from day to day, and secondly, to constantly raise the level of quality for a product. For the immediate

success of the business, the first is extremely important, but for the long pull, the second may be equally as important.

5. It was agreed that the ADSA score cards for products were helpful in appraising quality and in gradually arriving at a more uniform standard of quality throughout the country.

Interest in the Symposium seemed to be very good indeed, and it was generally agreed that a Symposium of this type might well constitute a part of the program of future meetings.

CHEESE AND EVAPORATED MILK

M12. The Gas Packing of American Cheddar Cheese.

N. S. GOLDING, J. N. REYNOLDS, AND C. C. PROUTY, State College of Washington.

The canning of cheese either in valve vent cans or vacuum packed has been under investigation for a number of years. This paper considers gas packing of degassed cheese with either CO_2 or N_2. Standard conditions for weight of cheese and space in the can were maintained in all experiments. 2041 grams of cheese known to produce little or no gas during ripening were sealed in No. 10 five-inch cans with a 1-mm. opening or a ⅛-inch tinned copper side arm to which a pressure rubber tube could be attached.

The cheeses were then degassed in desiccators for various lengths of time at room temperature and at an absolute pressure of from 20 to 30 mm. of Hg. Following degassing, CO_2 or N_2 was rapidly filled into the desiccator returning it and the can to atmospheric pressure. The opening in the can was immediately sealed by soldering or the tube closed with a pinch clamp. The loss in weight due to degassing even up to 72 hours was insignificant. Subsequent changes of pressure in the can of cheese were then measured on a Hg manometer or by a pressure can tester.

With CO_2 gassing a pressure equilibrium was reached in about four days. Cheese degassed for 1 hr., 24, 48, and 72 hrs. had a vacuum in the cans of 355, 390, 394, and 401 mm. of Hg, respectively. Cheeses of the same batch and weight cut into ½″ slices and degassed for 24 hours reached a pressure equilibrium much sooner and had a vacuum of 465 mm. of Hg. Reducing the space in the can by using more cheese increased the ultimate vacuum. When N_2 was used to gas the cheese subsequent to similar degassing only a slight vacuum was produced in the can of cheese.

M13. The Action of the Associate Starter Organisms in American Cheddar Cheese.

C. C. PROUTY AND N. S. GOLDING, State College of Washington.

Cheese starters as they are commonly used are not pure cultures of lactic-acid-producing bacteria. Rather they are mixed cultures, containing in addition to the lactic-acid-producing organisms the so-called associate types *S. citrovorus* and *S. paracitrovorus*.

Since these organisms are capable of producing CO_2 a study was planned to determine the extent of the vacuum changes occurring during the ripening process of vacuum-packed American cheddar cheese to which known quantities of either *S. citrovorus* or *S. paracitrovorus* or both were used in conjunction with either *S. lactis* or *L. bulgaricus* or both in the cheese starter.

Cheese in which the starter consisted of *S. lactis* or *L. bulgaricus* alone or in combination showed little vacuum loss at ripening temperatures of either 45° or 55° F. over periods ranging up to six months, whereas when *S. citrovorus* or *S. paracitrovorus* were used in conjunction with the lactic-acid-producing starter organisms a greater loss of vacuum occurred, more loss being associated with the use of *S. paracitrovorus* than with *S. citrovorus.*

M14. Studies on the Resistance of Various Cheese Cultures to the Action of Bacteriophage. F. J. Babel, Dairy Industry Department, Iowa State College.

In an attempt to eliminate slow acid production due to bacteriophage during the manufacture of cheese, a system of culture rotation was practiced. This rotation included six cultures which were being carried in the regular culture collection in the Dairy Department of Iowa State College and one culture obtained from a creamery organization. None of the seven cultures were sensitive to the bacteriophage strains which had been responsible previously for slow acid production during the manufacture of cheese. The rotation was arranged so that the same culture would not be used more than one day per week. This arrangement was quite satisfactory for one week. During the second week, four of the seven cultures produced acid slowly due to the appearance of bacteriophage active against these cultures. In the third week, all of the cultures were slow and bacteria-free filtrates active against all of the cultures were obtained from samples of whey.

When slow acid production during cheese manufacture was first encountered, the bacteria-free filtrates prepared from the whey, obtained from vats in which acid production was slow, were active only against the culture being employed at that time. Also, when the system of culture rotation was first practiced, the bacteria-free filtrates containing bacteriophage were active only against the culture employed in the cheese-making operation. During the second and third weeks in which the system of culture rotation was practiced, bacteria-free whey filtrates active against two or more cultures were obtained. Bacteria-free filtrates prepared from single plaque isolations of bacteriophage were active also against one or more cultures.

Several cultures of *Streptococcus lactis* isolated from sour cream and sour milk were not sensitive to any of the bacteria-free filtrates containing bacteriophage which were responsible for previous instances of slow-acid production. One culture of *S. lactis* (W4) isolated from sour cream was used in the cheese laboratory for approximately 3 months before it was attacked by bacteriophage. Another culture of *S. lactis* (W8) obtained from the same sample of cream as culture W4 was sensitive to the bacteria-free filtrate prepared from the whey in the vat in which culture W4 was used. A culture of *S. lactis* obtained from sour milk was used in the cheese laboratory for 5 months; at the end of this time the culture was still very satisfactory but was discontinued in order to study certain other cultures.

The use of *S. lactis* cultures isolated from certain natural sources appear to be an immediate solution to the bacteriophage problem in cheese plants experiencing difficulty with slow acid production.

M15. The Influence of the Quality of Milk and the Curing Temperature on the Ripening of Pasteurized-Milk Cheddar Cheese.

GEORGE P. SANDERS, HOMER E. WALTER, AND RALPH P. TITTSLER, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Quality-improvement experiments, including ripening studies, were conducted on approximately 260 vats of Cheddar cheese cured at different temperatures from 40° to 70° F. Among these, 18 lots of cheese were made by the time-schedule method from raw and pasteurized portions of milks of good, fair, poor, and very poor quality. Samples from separate daisy-size cheeses, cured at different temperatures, were graded at definite ages and tested also to determine their chemical, bacteriological, and physical properties.

In making cheese from pasteurized milk by the time-schedule method, it was comparatively easy to control the acidity within a range favorable for the production of the best cheese—*e.g.*, titratable acidity of whey 0.45 to 0.50 per cent, pH of curd 5.35 to 5.40, at milling. It was much more difficult to control the acidity in making cheese from raw milk, especially with low-grade milk, and excessive acidity during making and ripening was commonly associated with poor quality of cheese.

The quality of the milk and the curing temperature had much greater effects, on the rate of ripening and on flavor, than did pasteurization. When the cheese was made from high-grade milk, pasteurization had little effect on either the rate of ripening or the rate of development of flavor. Proteolysis was much more rapid in raw-milk cheese made from poor milk than in that made from good milk. The extent of proteolysis in cheese cured at 50° F. for 6 months (*e.g.*, approximately 30 per cent of the nitrogen soluble) was comparable with that in cheese cured at 60° for 3½ months.

The cheese made by the time-schedule method from pasteurized milk was consistently better and more uniform in quality than that made from raw milk, except when very high-grade milk was used. The improvement in the quality of the cheese resulting from pasteurization was greatest when medium-grade and low-grade milk, especially the latter, was used. Proper pasteurization suppressed completely the defect of gassiness.

The cheese made by the time-schedule method from high-grade, pasteurized milk and cured at 60° F. developed not only more but also better flavor than the control cheese cured at 50° or lower. On the other hand, when low-grade milk was used, either raw or pasteurized, the quality of the cheese cured at 60° was inferior to that of the cheese cured at 50°, and this inferior quality was especially pronounced when the low-grade milk was not pasteurized. A curing temperature of 70° was found to be too high even when high-grade milk was used.

M16. A Grating Type Cheese. G. H. WATROUS, JR., AND C. D. DAHLE, Pennsylvania State College.

Imports of highly flavored, dry cheese of the Parmesan type have been drastically curtailed. Prior to the war, Italy was the main supplier of this type of cheese, so popularly used with spaghetti, soups, and in many foods as a condiment.

The characteristic flavor of some types of grating cheese, imported or domestic, is of free fatty acid similar to butyric acid. In the imported cheese, the characteristic flavor as well as the hard dry body is obtained only after curing from one to three years.

The authors conceived the idea that a type of cheese suitable for grating could be obtained in a much shorter time by forcing the development of the fatty acid flavor by means of homogenization.

It was found that a good, characteristic flavor, body and texture could be obtained from raw skimmed milk which had been standardized to two per cent fat with homogenized pasteurized cream. The lipase in the raw skimmed milk produced the free fatty acid from the fat of the homogenized pasteurized cream.

Control lots were made using rennet paste, but very little flavor developed within a year, while the cheese made from raw skimmed milk and homogenized pasteurized cream had a good flavor in five months and an excellent flavor and body in seven months.

M17. The Influence of Age and Degree of Ripeness on the Activity of Cheese Cultures. H. C. OLSON AND RUSSELL BEACHBOARD, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.

Ripened cheese cultures held at 40° F. and 50° F. showed no significant decreases in activity during storage for five days; the cultures were removed from storage, inoculated into pasteurized milk, and the rate of acid production at 86° F. determined. These cultures showed marked decreases in activity when held beyond five to seven days. The cultures held at 32° F. and below freezing (0° F. to $-10°$ F.) showed marked decreases in activity during storage. When the cultures which had been stored at the various temperatures for different periods were removed and run through one intermediate transfer in milk at 70° F. for sixteen hours before determining their activities, they were much more active than when the original stored cultures were inoculated directly into the milk. When this intermediate transfer was employed there were no significant differences in the activities of the cultures stored at 32° F., 40° F. and 50° F. during the first five days, but beyond five days the influence of age in slowing up the cultures was apparent. The cultures which had been held frozen and then passed through an intermediate transfer still reflected the influence of the damaging effect of the freezing.

The degree of ripeness of the culture at the time of inoculation into cheese milk apparently has no significant influence on the activity of the culture, providing the culture was firmly coagulated at the time of its use. Ripening for several hours beyond the usual ripening period appeared to have no detrimental effect on the activity of the culture. Our results indicate that for best results the cheese culture should be freshly prepared and thoroughly ripe.

M18. A Study of the Physical Changes Which Affect the Storage Life of Evaporated Milk.

B. H. WEBB, E. F. DEYSHER, AND C. F. HUFNAGEL, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Important changes which sometimes occur during prolonged storage of evaporated milk include a separation of fat, of protein, and of insoluble salt crystals, as well as a darkening in color. Many factors control the magnitude of these changes, including conditions of manufacture and of storage. Most commercial evaporated milks remain in a good physical state after 2 to 3 years' storage under favorable conditions. Occasionally some undesirable changes occur after storage for 6 months or longer and these are more pronounced under adverse storage conditions.

The viscosity of all evaporated milks dropped to a basic level during storage. The relationship between the time required for a milk to thin to such a level and the storage temperature was logarithmic with respect to time. The magnitude of the change in viscosity during storage varied for different milks. A stable high-grade milk with an initial viscosity of 55 centipoises had a viscosity of 27 centipoises after 200 days' storage at 86° F. One of the poorer milks held under the same conditions dropped from 66 to 16 centipoises.

There was a tendency for the protein of some evaporated milks to settle to the bottom of the can during storage. This protein separation was more pronounced in evaporated skim than in evaporated whole milk. Homogenization retarded protein separation in evaporated whole milk. The results indicated that the fat in evaporated milk was very important in helping to maintain a normal dispersion of milk constituents throughout the can during long storage periods. The buoying tendency of the fat seemed to counterbalance the settling tendency of the protein.

The size of the fat globules in evaporated milk as measured by the Farrall Homogenization Index gave an indication of the fat separation which could be expected in poorly homogenized milks of known viscosities.

A linear relationship existed between the time and temperature of sterilization of evaporated milk in respect to the development of color over the temperature range 225° to 255° F. The amount of color that developed during the storage of evaporated milk at various temperatures was logarithmic with respect to time.

Analysis of the small hard crystals which formed in some samples of evaporated milk after storage for 2 to 3 years showed the crystals to be largely calcium citrate. When milks were seeded with calcium citrate or when solutions of calcium chloride and sodium citrate were added to milks before sterilization, the growth of crystals during storage was accelerated.

M19. Observations on the Judgment of Flavor Defects in Dairy Products. D. V. Josephson, Department of Dairy Technology, Ohio State University.

In the training of students and in the selection of tasting panels for scoring dairy products, little consideration has been given to the determination of the individual taste patterns of the judges and their abilities to detect and evaluate flavor defects. Individuals vary widely in their abilities for detecting basic flavor qualities as well as deteriorative defects in dairy products.

A study connected with five trained observers (three of whom had judged dairy products for 15 to 25 years) demonstrated that each individual has a definite taste pattern which is rather constant from day to day. However, an observer, who is sensitive to certain taste qualities, may require relatively high intensities of others to give consistent reports.

Working with U.S.P. lactose in varying volume-molar concentrations the five observers demonstrated taste thresholds (points or ranges where significant reports of "sweet" were given) of 0.05 to more than 0.133 volume-molar. In other words, one observer consistently detected a sweet quality in a solution containing 1.8 grams of lactose per liter, while another required in excess of 48 grams per liter. The addition of 0.15 per cent salt (NaCl) to a solution containing 4.8 per cent lactose (normal concentration for milk) intensified the sweet quality of the solution remarkably. One observer, who could not detect sweet in a 4.8 per cent lactose solution, consistently reported "sweet" when the salt was present. Another observer consistently reported "salt-followed-by-sweet" for such a solution. This observer later demonstrated an extremely low threshold for salt. Salt

(NaCl) threshold values for the five observers varied from 0.0025 to 0.02125 volume-molar, and those most capable in detecting salt were the least capable of detecting the sweet quality of lactose. Dilute solutions of salt (below threshold level) were usually sweet to most observers while lactose was frequently bitter or sour at such levels. At certain concentrations salt solutions tasted ''salty'' to some observers while others consistently reported ''sweet'' or ''flat'' on the same solution. Similar relationships exist in the detection of ''cooked,'' ''oxidized,'' ''rancid,'' ''salty,'' and other defects in milk. For example, carefully controlled experiments where the factors of size of sample, temperature of sample, and interval between samples were controlled, one judge consistently described a flavor defect as ''pronounced,'' another described it as ''slight,'' while another could detect no off-flavor. An observer most sensitive to one flavor defect was frequently least capable of detecting another.

The tactual taste qualities (from touch response of tongue and mucous membranes) were found to be of extreme importance in certain dairy products. In powdered milks, tactual flavor qualities are very prominent and are described as ''powdery,'' ''chalky,'' ''rough,'' ''astringent,'' or ''puckery.'' On a given sample of re-constituted powdered milk, the five observers varied in their description of the sample depending upon the sensitivity of their tactual responses. Some observers were extremely sensitive to the tactual quality and described it as ''puckery'' or ''astringent'' while others, who were less sensitive to it, reported ''chalky'' or ''powdery.'' Very fresh milk powders and old milk powders appeared to exhibit the most prominent tactual qualities. Evaporated milk exhibited similar but more intense tactual flavor qualities. Although insufficient evidence is available at this time, it is probable that sulfides or other protein degradation products, or a combination of these with lactose degradation products are responsible for this defect in powdered and evaporated milks.

Because of the great variation among observers in their abilities to detect and evaluate flavor qualities and defects, it would appear that greater care might be taken in setting up judging panels for tasting experimental samples and in the training of students in the grading of dairy products. Furthermore, a more standardized system of terminology and score should be adopted, particularly for milk. The present scoring ranges applied to milk flavors are far too wide to permit uniform judging even among trained and experienced judges.

BACTERIOLOGY AND CHEMISTRY

M20. A Water-Soluble Antioxidant for Milk and Milk Products.

N. P. TARASSUK AND J. L. HENDERSON, University of California, Davis, California.

During the last four years an increasing emphasis has been placed on antioxidants for preservation of fats, oils and fat-containing foods from oxidative deterioration. Milk, cream, whole milk powder and other milk products are subject to either spontaneous or the copper-induced oxidized flavor. In winter when cows are fed dry feed, certain cows produce milk highly susceptible to oxidation and the milk and cream will develop an oxidized flavor on the second or third day in spite of good processing practices. The use of a suitable antioxidant offers a practical solution in these instances.

To be used in a dairy product, an antioxidant must be entirely innocuous, impart no color, flavor or odor to the product, remain stable during processing treatments, and it should be sufficiently soluble in the product to make its incorporation uniform and easy. The last requirement necessitates that an antioxidant for milk must be water-soluble because the use of other solvents, say alcohol or acetone, would be neither safe nor practical. The better-known antioxidants at the present are not water-soluble.

In 1943 the authors reported in the paper entitled ''Vitamin-B-Complex Preparations as Antioxidants in Milk and Milk Products'' that the source of antioxygenic activity of these preparations resides not in the known crystalline B vitamins but in the materials used as ''carriers,'' flavoring materials, or the concentrates from natural sources which contain certain vitamins. This material was fractionated by means of various solvents and the fractions obtained were tested for the antioxygenic activity. Two groups of compounds were isolated as possessing antioxygenic activity and identified: 1. The tannins; 2. The flavones. Both groups are widely distributed in many species of plants used for animal-human consumption. The flavones are naturally occurring plant pigments responsible for a yellow color of many flowering plants and are commonly found in citrus fruits. Tannins are present in many food plants, such as, asparagus, sweet almonds, linseed, caraway seeds, plums, other fruits and coffee. Tea contains on an average of twelve per cent tannin.

Experiments with C.P. tannic acid and quercetin (tetrahydroxy flavone) have shown them both to be effective in prevention of the development of either spontaneous or the copper-induced oxidized flavor in milk and cream when they were added in a concentration as low as 0.0025%. Addition of 0.01% of either material prevented the development of oxidized flavor in milk susceptible to ''spontaneous'' oxidation to which 5 p.p.m. ofCu^{++} had also been added.

Incorporation of quercetin in milk is difficult since it is not water-soluble. Tannic acid, on the other hand, is water-soluble. Its incorporation in milk or cream is easy and simple. Small amounts that are required can be dissolved in few ml. of water or added directly to milk or cream. Tannic acid, even when added in the amount several times as great as necessary, does not impart any flavor foreign to the milk. The wooden musty odor of tannic acid which occurs in some commercial lots is removed entirely by heating water solution of tannic acid to boiling before it is used. Addition of tannic acid to milk in concentration of 0.005% or higher before drying prevents the oxidative deterioration of milk powder. Tannic acid was found to be also a very effective antioxidant for milk fat, lard and other animal fats when added to these products in concentration of 0.01% or higher. Glycerol can be used as a solvent for incorporating tannic acid in fats and oils. The water extract of tea has greater antioxygenic activity in milk than can be accounted on tannin basis. An addition of 0.20% by volume of the 5% aqueous extract of tea was sufficient to prevent the development of oxidized flavor in milk to which 0.5 p.p.m. of Cu^{++} was added.

Patent applications have been filed, Serial No. 542, 596 and No. 542, 597, June, 1944, for the use of tannic acid and quercetin in milk and milk products and tannic acid in animal fats.

M21. Isolation of Whey Proteins and Their Conversion into Food Products.

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Only one-fourth of the whey resulting from cheese-making in the United States is utilized as human food. The remaining 7 billion pounds of whey, which contains 70 million pounds of protein of high nutritional value for humans, is utilized as feed for animals or is wasted.

The increased demand for food during the war, followed by the great need for providing additional sources of food at the present time, has focused attention on methods for conserving this highly nutritional protein.

Our research has resulted in improved methods for recovery of whey proteins, which are readily adaptable for use in any cheese factory. These methods consist of heating the sweet whey (after adjustment when necessary to pH 6.4 or to a titratable acidity of between 0.11 to 0.14 per cent lactic acid) to near boiling temperature by means of a steam injector heater; precipitation of the proteins by the addition of sour whey or acid to lower the pH of the heated whey, preferably to between 4.8 and 5.3; and recovering the protein curd by dipping, as in making Swiss cheese, or by draining off the whey filtrate as is the practice in making Cheddar cheese.

The protein curd obtained from Cheddar cheese whey is likely to be firmer and more grainy than that obtained from Swiss cheese whey. Precipitation at temperatures above 205° F. likewise yields a firmer, more grainy-textured curd. On the other hand recovery of the precipitated protein is more difficult when precipitation occurs below 200° and when too much agitation is used during the precipitation process.

After complete drainage of the whey, the protein curd has a pleasant but not a pronounced flavor; the acidity is between pH 5.6 and 6.0; it contains about 75 per cent moisture, 16 per cent protein, and 2.5 per cent fat; it is slightly grainy but mats well so that it can be milled and pressed in hoops as is Cheddar cheese curd; and it is practically sterile but mold and bacteria grow well on it.

Studies have been carried out in the conversion of the protein curd into cured cheese, cottage and cream cheese, and cheese spreads. *Penicillium roquefortii* brings about a rapid break-down of the curd when it is used in making a Roquefort-type cheese. This curd has shown promise also when made into a cottage cheese. The grainy texture of the curd interferes to some extent with its use in the making of cream cheese and cheese spreads.

M22. Variations in the Acid Degree of Milk Fat as Affected by Churning and Extraction Procedures.

B. C. JOHNSON AND I. A. GOULD, Maryland Agricultural Experiment Station.

Since preliminary studies indicated that the free acid content of fat was higher when the fat was obtained from cream by solvent extraction than when it was obtained by churning, additional experiments were conducted to study this relationship more thoroughly and to determine the applicability and reliability of the respective methods. One phase of the study consisted of developing a suitable extraction procedure. In this investigation, various proportions of the solvents; *i.e.*, ethyl ether and Skellysolve F, were used to determine the most satisfactory ratio. The procedure adopted was as follows: Rancid cream was produced by homogenizing raw cream of 30–32 per cent fat, holding several hours and then heating to 145–150° F. for 10 minutes to inactivate the lipase. One hundred twenty-five grams of this cream were shaken for 15 seconds with 100 ml. of ethyl alcohol and allowed to stand 5 minutes. To this mixture was added a total of 200 ml. of the extractants, the ratio of ethyl ether to Skellysolve varying as follows: 4 : 0, 3 : 1, 1 : 1, 1 : 3, and 0 : 4. In one series of trials, the respective solvents were combined prior to their addition to the sample and this mixture was incorporated into the sample by a shaking period of 30 seconds. In another series, the solvents were added separately to the cream-alcohol sample with 15 seconds shaking after each addition. Following the addition of the solvents, the mixture was centrifuged for 3 minutes at approximately 1500 r.p.m., the supernatant ethereal portion removed by syphon and the solvent evaporated. Portions of the same cream used for solvent extraction were also churned according to standard procedure. Free fatty acid content of the fat was determined by titration of a 10-gm. sample with N/10 NaOH to the phenolphthalein endpoint.

The resulting data reveal that the most satisfactory ratio of ethyl ether to Skellysolve was approximately 1 : 1 but that this proportion may be varied to some extent. Slightly higher and more uniform titration values resulted when the solvents were mixed before being added to the cream than when they were added separately. When the ratio of the ether to the Skellysolve was 1:1, the solvent extracted fat averaged about 30 per cent higher in titration value than did the churned fat although this varied somewhat with the individual trials.

Studies were also made to determine the effect of such factors as the presence of formalin and lactic acid in the cream on the free acid content of the fat obtained by the extraction and churned methods. When formalin was added at the rate of 1 ml. per pound of cream it was found to reduce the efficiency of fat recovery both by extraction and by churning, and to result in a titration increase of about 8 per cent in the churned fat. The addition of 0.2–0.3 per cent of lactic acid to the cream resulted in appreciably higher titers of both extracted and churned fats: the extracted fat increasing about 4 per cent and the churned fat about 27 per cent in comparison to the control.

Applications made of the extraction procedure indicate that it may possess definite advantages over the churning method for measuring the degree of fat splitting which results from lipase activity.

M23. Concentration of the Free Fatty Acid Portion of Milk Fat by Alcohol Extraction. I. A. Gould, Maryland Agricultural Experiment Station.

In studies conducted to ascertain the specific changes which occur in milk fat as a result of lipase action, steps were taken to concentrate the free fatty acid portion of the fat by means of hot ethyl alcohol extraction, and then to determine the fat characteristics of this alcohol-soluble fraction. Although several procedures were attempted, the one finally adopted was as follows: Butter-oil was heated to boiling in approximately twice its volume of ethanol, the mixture centrifuged, and the supernatant liquid largely removed by siphoning. This treatment with hot ethanol was repeated twice and the three lots combined. The alcohol extract was cooled overnight at 32–35° F. and the small quantity of white precipitate was removed by filtration. This white precipitate contained no appreciable acidity. The alcohol in the clear filtrate was then removed by heating under a partial vacuum, the fat taken up in ether and Skellysolve F and the solvent removed under partial vacuum. The final extract was liquid at room temperature, golden yellow in color, and contained more than 90 per cent of the free fatty acids of the original fat. The extract from non-rancid fat was practically devoid of any rancid flavor whereas the extract from the rancid fat possessed a pungent bitter flavor. The yield of extract averaged 7.8 g./100 g. of non-rancid fat and 20.2 g./100 g. of rancid fat. On the basis of preliminary trials and when compared to the original fat, the extracted material from non-rancid fat exhibited the following approximate changes: a nine-fold increase in acid degree, a two-fold increase in Reichert-Meissl and Polenske numbers and in soluble acids, a 10 per cent increase in the saponification and in the iodine numbers, a 10 per cent decrease in the non-soluble acids, and a slight decrease in the refractive index.

The extract obtained from rancid fat exhibited values comparable to those of the extract from non-rancid fat, but showed somewhat smaller increases in acid degree, in Reichert-Meissl and Polenske numbers, and in the soluble acids when compared to the original fat. The data obtained from these preliminary trials by the alcohol extraction procedure do not permit a clear differentiation between rancid and non-rancid fat on the basis of the fat characteristics of the resulting extracts nor an identification of the specific fatty acids involved in lipolysis.

M24. The Direct Microscopic Count as a Method for Counting Bacteria in Pasteurized Milk. M. P. BAKER, Iowa State College, Ames, Iowa.

Studies were made of the effect of pasteurization on the staining of bacteria in milk by the direct microscopic technic. Raw milks with low initial bacterial counts were inoculated from skimmed milk cultures of types of bacteria commonly found in milk. The cultures from which these were taken were incubated at 20° and 37° C. and for 24, 48 and 72 hours respectively to observe the effect of temperature of growth and age of culture. Direct microscopic counts were made on the raw milks immediately after addition of the bacteria and again immediately after pasteurization and cooling, pasteurization being at 143° F. for 30 minutes. The pasteurized samples were then held for 72 hours at approximately 3° C. and direct counts made at 24-hour intervals to determine if there were any changes in the ability of the cells to stain. Organisms used included *Streptococcus lactis, Streptococcus durans*, several strains of micrococci, several strains of aerobic spore formers, *Lactobacillus casei, Pseudomonas putrefaciens* and *Alcaligenes viscosus.*

The pasteurized milk counts expressed as per cent of the companion raw milk counts varied considerably with all types of organisms studied. When *S. durans* was used as the test organism the pasteurized counts averaged almost as high as the raw milk counts. There were some noticeable exceptions, however, and in some trials the counts were much lower after pasteurization. This was true with the micrococci also. With *S. lactis* generally a somewhat lower percentage of the cells stained after pasteurization, in the majority of the comparisons the pasteurized milk counts being less than 60 per cent of the raw milk counts. The other organisms studied stained noticeably poorer and the *Ps. putrefaciens* showed especially poor staining qualities after pasteurization.

The results of these studies indicate that the direct microscopic count on pasteurized milk may or may not be equal to a similar count on the same milk before pasteurization. In some instances it may be only a small fraction of what the direct count on raw milk had been. These differences apparently depend on the types of bacteria present in the milk, on errors in technic and on other factors not indicated by the data obtained.

M25. Non-Lactose Fermenting Yeasts and Yeast-Like Fungi from Cream and Butter.

STANLEY H. F. CHINN AND F. E. NELSON, Iowa State College.

Three hundred and sixty-nine yeasts and yeast-like fungi were isolated from 124 samples of cream and 203 samples of butter. Of the 342 cultures which were unable to ferment lactose, 3 were species of *Saccharomyces,* 41 of *Rhodotorula,* 9 of *Pullularia* (tentative), 81 of *Torulopsis,* 11 of *Trichosporon,* and 197 of *Candida.* Twenty-eight types were recognized of which 14 could be identified quite definitely with known species and 6 differed only in minor characteristics from recognized species. Upon the basis of correlated characteristics, 8 types were differentiated from previously described species but new specific designations were not given to these pending further comparative study.

The heat resistance of the organisms isolated was low; only a few strains in 3 types resisted 61.7° C. for 5 or 10 minutes and all were destroyed by 30 minutes at this temperature.

The defects produced by representative cultures of the various types when inoculated into cream or unsalted butter ranged from very pronounced off-flavors to no off-flavors. Bitterness and proteolytic and lipolytic defects were of the greatest significance, although a number of other types were encountered at the less-serious level. The presence of *S. lactis* enhanced the development of off-flavors in many cases.

The widespread distribution of non-lactose-fermenting yeasts and yeast-like fungi in cream and butter and their ability in many cases to cause defects indicate they may be of significance under some circumstances in the deterioration of these dairy products.

M26. Improvements in the Phosphatase Test Applied to Milk and to Cheese.

GEORGE P. SANDERS AND OSCAR S. SAGER, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

A modification of the phosphatase test for detecting under-pasteurization of milk has been developed for application to Cheddar cheese, and this modification has been applied also in testing fluid milk.

Research on the conditions and substances that cause interference in the test has been conducted, and the modifications found effective in minimizing the interferences are described. The resulting test has been found to yield results that are more precise for detecting under-pasteurization, both in milk and in Cheddar cheese, than those obtained with the tests available heretofore.

In tests on whole milk and on non-ripened cheese, contamination of the pasteurized milk with as little as 0.05 per cent of raw milk (1 pound in 2,000) can be detected. The test applied to cheese has been found reliable for detecting under-pasteurization adequately, regardless of the age of the cheese.

M27. The Relationship of the Growth Rates of all Bacteria and Coliform Bacteria in Pasteurized Milk Held at Refrigeration Temperatures.

A. C. DAHLBERG, Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In a study of the milk supply of New York City, it was found that the coliform bacteria in pasteurized market milk held at refrigeration temperatures increased more rapidly in numbers than the total count. In the freshly pasteurized milk less than 0.02 per cent of the total bacteria were coliform types. At 35–40° F. the percentage of the total bacteria which were coliform did not increase in October, but in July and August increased to 1.12 per cent in 4 days. After storage for 4 days at 45–50° F. and at 55–60° F. the coliform bacteria constituted about 5 per cent of the total count in October and 88 and 50 per cent of the total count in July and August. The coliform bacteria grew relatively more rapidly in warm weather than in cool weather.

CHEMISTRY, SANITATION, TEACHING

M28. Some Observations Regarding the Effect of Various Wave Lengths of Light on the Riboflavin Content and Flavor of Milk.

D. V. JOSEPHSON, Department of Dairy Technology, Ohio State University.

A series of specially constructed glass exposure cells were made to facilitate a study of the effect of various wave-length ranges of light on the riboflavin content and flavor of milk. The cells were constructed so as to permit the mounting of various light filters on the exposure surface. Each cell was divided into three separate compartments by glass plates. The compartments varied in depth from 0.12 to 0.4 inch and were in no way connected with adjacent sections. The cells, including the cell dividers, were made of "water-white" plate glass cemented together with Vinylseal. All surfaces except that exposed to light were covered with black oilcloth so that light rays entering the first compartment from the exposure surface had to penetrate three plates of glass and from 0.32 to 0.52 inches of milk (depending on cell construction) to reach the milk in the back compartment. In most of the studies, the cells were arranged so that the 0.12-inch compartment was directly exposed to the light. Riboflavin content and flavor development were measured or observed at different periods of exposure in all compartments.

When a control cell (without light filters) was exposed to direct sunlight (200–400 Weston degrees), the riboflavin loss in the exposed compartment (0.12-inch layer of milk) was extremely rapid. A 70 per cent destruction of this vitamin was not uncommon in the exposed compartment after 20 minutes exposure. After this time, the rate of destruction decreased but after one hour losses ranged from 93.0 to 99.0 per cent. The second compartment (0.2-inch layer of milk) immediately behind the exposed section suffered riboflavin losses varying from about 5.0–10.0 per cent after 20 minutes to 33.0–45.0 per cent after one hour exposure. In the back compartment (0.4-inch layer of milk), no loss of riboflavin resulted in any of the trials. However, when the cell was reversed and the 0.4-inch compartment was made the exposure surface, the loss was 40.0–50.0 per cent in 20 minutes and 80.0–90.0 per cent in one hour. With this compartment used as the exposure surface, no loss of riboflavin was experienced in the middle (0.2-inch) or back (0.12-inch) compartments. From this, it would appear that the rays responsible for riboflavin destruction are incapable of penetrating more than about 0.3 inch of milk.

Trials in which various light filters were adapted to the exposure surfaces of the cells containing homogenized milk demonstrated that the wave lengths of light responsible for the photolysis were those below 520 mμ length (5200 Å). Filters, which cut out all wave lengths below 430, 450, 475, 490 and 500 mμ failed to prevent riboflavin loss, but those which cut out wave lengths of light below 490–500 mμ, afforded fair protection. For example, in one experiment where the milk in the control cell showed a 98.9 per cent loss in the exposed compartment after one hour of exposure, that in similar cells fitted with filters which cut off at 490 and 500 mμ suffered riboflavin losses of 7.1 and 5.4 per cent, respectively. Numerous other filters, which cut out at higher ranges (520–740 mμ), gave complete protection to riboflavin.

The development of "sunlight" flavor in milk exposed in these cells presented an interesting but different picture. To prevent the development of "sunlight" flavor in the milk in the exposed cell, a filter which eliminated all wave lengths below 620 mμ was required. With certain milks and with very strong sunlight, a slight flavor developed even with this filter. A filter, which eliminated all wave lengths below 740 mμ, gave complete flavor protection. Milks exposed in cells fitted with filters, which cut off at 530, 550, 570, and 590 mμ, developed the same degree of "sunlight" flavor as the milks in control cells without filters. This would suggest the possibility that the rays responsible for "sunlight" flavor development are those in the 590–630 mμ band. Because of the inavailability of a complete range of filters cutting off above 620 mμ, the maximum point for complete protection under all conditions could not be determined.

The longer wave lengths of light, which are responsible for the sunlight flavor, have greater penetrating powers than those responsible for riboflavin photolysis. In all trials (without light filters), the "sunlight" flavor developed in all compartments of the cells. By the use of the varying-depth cells, it was demonstrated that these wave lengths could penetrate three plates of glass (compartment dividers) and 0.6 inches of milk and produce the flavor in the back compartment.

The development of "sunlight" flavor is undoubtedly a very complex oxidation-reduction process. In some studies with special cells, the flavor developed very rapidly in the exposed compartment, but on further exposure disappeared almost entirely. In some experiments the milk in the exposed compartment exhibited a strong sunlight flavor after 15 minutes of exposure, while that in the middle and back compartments showed somewhat less. However, after one hour, the degree of flavor development was the reverse, with the back cell exhibiting the strongest flavor.

Freshly pasteurized milk developed the "sunlight" flavor more quickly and to a greater degree than did the same milk after aging. After 3–6 days of storage in a refrigerator, it was frequently difficult to produce any degree of "sunlight" flavor in milk even with long-time exposure. In a few cases, an "oxidized" flavor developed in these exposed aged milks.

Although the evidence is inconclusive at this time, it would appear that the "sunlight" flavor in fresh pasteurized milk (without metallic contamination) may be the result of the production of a complex reducing substance which in turn may actually have an anti-oxygenic effect on the oxidizable lipids.

M29. Sanitization of Creamery Water Supplies with Various Bactericides.

C. JENSEN, Department of Dairy Husbandry, North Dakota Agricultural College, Fargo, North Dakota.

Bacteriological investigation of 148 samples of creamery water revealed that 80 per cent of them contained sufficient numbers of fat- and protein-decomposing bacteria to be of importance in regard to butter deterioration.

Studies were made of various bactericidal agents including sodium hypochlorite and various surface-active compounds from the standpoint of sanitizing creamery water supplies. The surface-active compounds investigated were cetyl pyridinium chloride (Ceepryn), alkyl-dimethyl benzyl ammonium chloride (Emulsept and Roccal), di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride (Hyamine 1622) and di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride (Hyamine 10–X). Results indicate that sodium hypochlorite and the surface-active compounds studied are highly effective germicides in the treatment of water which has previously been filtered to remove organic residue and with the pH adjusted so as to obtain the most efficient action from the bactericide. The bactericidal action of sodium hypochlorite decreased rapidly with increased concentration of hydroxyl ions in water. It was also affected materially by the presence of organic matter in the water.

With the exception of Ceepryn, the bactericidal activities of the surface-active compounds were affected to a marked degree by variation in the pH level of the solutions.

Lethal doses of the various bactericides were employed for washing butter granules without producing appreciable signs of flavor defects in butter.

M30. The Manufacture of Powdered Cream-Mix for Whipping by Aeration.

HARRY PYENSON AND P. H. TRACY, Department of Dairy Husbandry, University of Illinois, Urbana, Illinois.

A new dried cream-mix has been developed for whipping by Aeration consisting of approximately 30 per cent butterfat, 7.5–8 per cent M.S.N.F., 5 per cent sugar and 0.2–0.3 per cent whipping agent on a reconstituted basis. This product when whipped by aeration produces a whipped cream product quite similar to that whipped from a fresh sweet cream-mix. Where it is often difficult to obtain fresh cream of the proper quality or where the supply is not adequate, this cream-mix can readily be stored in powder form and used when needed.

The cream-mix may either be dried with or without sugar. The sugar can be mechanically mixed with the powdered cream or added to the water at the time of reconstitution. Large or small nozzle size does not seem to affect appearance or whipping ability of the reconstituted powdered cream-mix. Homogenization pressure is detrimental to the whipping properties of the reconstituted cream-mix.

The keeping quality of the dried cream-mix can be improved by packing in inert gas and by the addition of certain antioxidants before drying. The most effective antioxidants of those studied are nordihydroguiaretic acid, gallic acid, and sodium Arabo ascorbate.

Powdered cream-mix produced in these experiments had an average composition of 68.8 per cent butterfat, 99.29 per cent total solids, 0.7 per cent moisture, 2.8 p.p.m. iron and 1.02 p.p.m. copper.

M31. Influence of Nonfat Dry Milk Solids on the Nutritive Value of Bread.

LLOYD K. RIGGS, ANNABEL BEATY, AND ARNOLD H. JOHNSON, Kraft Foods Company, Chicago, Illinois, and National Dairy Research Laboratories, Baltimore, Maryland.

The nutritive values of several breads were determined by feeding bread crumbs supplemented with cod-liver oil to growing rats for eight weeks and noting the number of grams of bread solids required to produce one gram gain in weight. The following types of bread were studied both with and without the addition of 6 per cent nonfat dry milk solids: (1) two lots of whole wheat bread, (2) white bread enriched according to the 1943 standards, and (3) white bread.

The addition of 6 per cent nonfat dry milk solids improved the nutritive value of all these breads. One lot of whole wheat bread had a higher than average protein content and an unusually high nutritive value. White bread containing 6 per cent nonfat dry milk solids was superior in nutritive value to enriched white bread both from the standpoint of growth promotion and chemical composition of the carcasses. Enriched white bread containing 6 per cent nonfat dry milk solids was equivalent in nutritive value to whole wheat bread of average composition as measured by growth promotion and carcass composition. On the basis of grams of bread solids required for a one-gram gain there was no difference between the nutritive values of enriched white bread containing 6 per cent nonfat dry milk solids and white bread containing 6 per cent nonfat dry milk solids.

M32. A Device to Aid in Determining the Effectiveness of Dairy Detergents. E. L. FOUTS AND T. R. FREEMAN, Florida Agricultural Experiment Station, Gainesville, Florida.

An experimental project is being conducted to determine the effectiveness of various brands of dairy detergents in washing dairy equipment and utentils on dairy farms and in dairy manufacturing plants. Water composition varies greatly throughout the state and a large number of washing powders are being offered for sale to the dairymen. It is not the purpose of this paper to report the results of the experiments on washing powders but to give information about a machine which was devised to aid in the washing trials. The machine has been named the Deterg-O-Meter.

The machine consists of a 16-inch metal bicycle wheel mounted in a pivoted frame which permits vertical movement, powered by an electric motor and geared to turn 7 revolutions per minute. A baked-on milk film is prepared on glass microscope slides under carefully controlled conditions. Eighteen of these slides with the milk film on them are then mounted on the rim of the wheel and held secure by metal clips. Water at a specified temperature, containing the required amount of washing powder is placed in a specially constructed pan. The wheel is lowered into the pan so that the slides dip into the water as the wheel turns and then are passed over a sponge rubber brush just as the slides emerge from the water. The slides are carefully observed and when clean they are removed from the wheel. The average number of revolutions required to clean the slides is taken as an index of the effectiveness of the washing powder. Other factors also are to be considered in evaluating the detergents.

M33. Some Experimental Results with a Centrifugal Separator the Bowl of Which Is Sanitized by Flushing Action. E. O. HERREID, R. V. HUSSONG, AND P. H. TRACY, Illinois Agricultural Experiment Station.

In 1938, H. W. Fawcett, of W. and J. Whitehead, Ltd., in Laisterdyke, England, brought a centrifugal separator to the Illinois Agricultural Experiment Station. This machine was similar to modern separators in construction except for certain features of bowl design. This bowl is designed to be cleaned by flushing action. It has a distributor which conducts washing solutions from the supply tank to all internal parts. In the shell of the bowl there are three outlet parts, equal distances apart. Each part is closed by a valve which is held in place by a steel spring. The centrifugal force closes the parts tightly when the bowl is revolving at normal speed. When the speed diminishes to about two-thirds that of normal, the valves open, and sanitizing solutions can be made to flow through the bowl with a turbulent cleansing action.

Some experiments were conducted with this separator in 1938. The results indicated that the principle of sanitizing the bowl without disassembling it daily was adaptable to farm use. The outbreak of World War II delayed further experimental work.

In July, 1945, an improved model of this separator was sent by H. W. Fawcett to the Illinois Agricultural Experiment Station. Ninety-eight experimental separations were made. The bowl of this English machine was cleaned by centrifugal flushing action, after each separation in the morning and in the evening, over periods of one week. Before each separation, water at 170° F., from the supply tank, was run through the tinware and the bowl. In the great majority of cases, there was no significant contamination from one separation to the next as revealed by plate counts of the cream and skim milk. The skim milk from the English machine averaged 0.08 per cent by the American Association test.

M34. An "In-Training" Course for Workers in The Dairy Manufactures Industry. P. H. TRACY AND E. O. HERREID, Department Dairy Husbandry, University of Illinois, Urbana, Illinois.

There is much evidence of increased interest in adult education on the part of both our rural and urban population. Dairy departments have an ever-increasing educational responsibility to the dairy industry. This obligation can be fulfilled in various ways, such as, short courses, conferences and in-training courses given by extension. The latter approach to the problem has been recently studied by the Dairy Department of the University of Illinois. Courses in Principles of Dairy Technology, Dairy Bacteriology, and Dairy Production have been given. Other courses in salesmanship, and supervisory training have been given by the University

Extension Division. In all, approximately 1,000 students have been trained during the past year in Chicago. Of these, 660 were registered in the beginning course in the Principles of Dairy Technology.

The qualification of those registered in the beginning course varied as indicated by the following summary.

I. *Educational Background*

100%	Attended grammar school
96%	Graduated from grammar school
80%	Attended high school
45%	Graduated from high school
21%	Attended college
5%	Graduated from college

II. *Age Distribution*

Age (years)	%
17–20	1.75
21–25	5.0
26–30	11.0
31–35	20.0
36–40	21.0
41–45	19.0
46–50	15.0
51–55	5.25
56–61	2.0

III. *Length of Experience (Dairy Industry)*

Period of time employed	%
No previous experience	55.5
1–12 months	4.5
1–2 years	5.0
3–5 years	8.5
6–10 years	6.25
11–15 years	4.5
16–20 years	7.75
21–25 years	4.0
26–31 years	1.75
Not indicated	2.25

IV. *Nature of work done*

Work done	%
Field work	2.0
Engineering	3.0
Supervisory	15.0
Plant Operation	20.5
Sales & Delivery	47.5
Office	9.5
Official & Owners	2.5

The students are registered by a representative of the Registrar's office. A fee of $10 per semester is charged. Classes are held once a week for 16 weeks at the John Marshall Law school. Length of class period—100 minutes. Periodic written quizzes and a final examination are given. Roll is taken at each meeting. A printed copy of all lectures is given each student. In one class of about 350 students, 68 per cent finished the course. The percentage attendance of those who finished was 94. The average grade in the course was 81.6. A certificate is given all who satisfactorily complete the course.

PRODUCTION SECTION

VITAMINS

P1. The Effect of Massive Doses of Vitamin D on the Blood Picture of of Dairy Cows at Parturition. J. W. Hibbs, Ohio Agricultural Experiment Station, Wooster, Ohio.

A study has been made of the effects of feeding large amounts of vitamin

D in the form of irradiated yeast and irradiated ergosterol in oil on the changes in serum calcium, phosphorus, and magnesium before and after freshening.

The feeding of 1 million units of vitamin D daily as irradiated yeast for 30 days before and 7 days after freshening did not cause any changes in the serum calcium, phosphorus, or magnesium of cows fresh normally or with milk fever as compared with control animals.

When 2 million units were fed according to the same plan an increase in serum calcium and phosphorus occurred before parturition.

When 5 million units of vitamin D in the form or irradiated ergosterol in oil (1 million units per gram) were fed daily for 14 days before the expected due date and for 3 days after parturition a still further increase in serum calcium was produced before freshening (12 mg. per cent as compared with 9.8 mg. per cent for the controls). However, regardless of the level to which serum calcium was raised before parturition the level within 12 hours after parturition was found to be between 9 and 9.5 mg. per cent in cows that did not develop milk fever.

In control cows that developed milk fever there was a tendency for the serum calcium and phosphorus to be markedly lower 12 hours before parturition than in control cows that freshened normally. When vitamin D was fed and milk fever developed there was a tendency 12 hours before parturition for the serum calcium to be relatively high, proportional to the amount fed. This increase was offset by a rapid decline after parturition until milk fever symptoms appeared.

Serum magnesium in nearly every case increased concurrent with the decrease in serum calcium in both normal and milk fever cows. There was no marked effect on serum magnesium as a result of feeding vitamin D.

The results of this study show that the feeding of vitamin D to cows before parturition will cause an increase in serum calcium and phosphorus before parturition. However, this increase is nullified within 12 hours after parturition since blood calcium is equal at this time in both vitamin D fed and control animals. Therefore a decrease in milk fever incidence is not to be expected from feeding as much as 5 million units of vitamin D in the form used and under the conditions employed, if the assumption is correct that, to prevent milk fever, blood calcium must be maintained near the normal level during the critical period. Incidence studies bear out this interpretation.

P2. Vitamin A in the Blood and the Liver of Calves, Neonatal and Colostrum-Fed.

G. H. Wise, M. J. Caldwell, F. W. Atkeson, and J. S. Hughes, Kansas Agricultural Experiment Station.

Past investigations of the prenatal nutrition of the dairy calf have been limited primarily to studies of the effects of deficiencies in the gestation diet of the dam. Recent observations indicate possible advantages of adding vitamin A in excess of the minimum necessary for the delivery of a "normal" offspring. Feeding massive amounts of vitamin A, approximately a million U.S.P. units daily during the latter stages of gestation, 8 to 45 days prepartal, effected a significant increase in the vitamin A reserves of the neonatal calf. The averages and ranges in values, γ/100 cc. serum and γ/gram of liver, respectively, were as follows for various prepartal rations fed: Standard winter, 2.3 (0–5.7) and 0.4 (0.2–0.7); standard winter plus pasture, 2.3 (0–4.7) and 0.5 (0.3–0.9), and standard winter plus vitamin A, 8.5 (5.7–11.3) and 5.0 (1.5–8.0).

The vitamin A levels of the blood serum dropped precipitously in the fasting newborn during the first 12 hours. The rate and degree of reduction varied with different individuals, the extremes in extent ranging from 29 per cent to 85 per cent for a 12-hour period. The relation of fasting to liver values have not been determined. Other factors affecting the early post-natal levels of vitamin in the newborn calf have not been explored.

The changes of the vitamin A values in the serum and the liver varied with the potency and amounts of colostrum consumed. The carotene and vitamin A concentration in the colostrum varied widely but reflected, in varying degrees, the vitamin A activity of the prepartal ration.

P3. The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine.

A. A. Spielman, J. W. Thomas, and J. K. Loosli, Department of Animal Husbandry, Cornell University, Ithaca, New York.

Four different rations were fed to 29 Holstein and 4 Guernsey heifers during the last 60 days of their gestation periods. The rations were: wheat straw plus a concentrate mixture, a normal fitting ration of concentrates, hay and corn silage, a normal ration plus one million I.U. of carotene daily, and the normal ration plus one million I.U. of vitamin A daily.

No significant differences were observed in the mean plasma carotene of the newborn calves from the four dietary groups. However, the plasma vitamin A of the newborn calves from the carotene supplemented cows was twice that of the normal group, while the vitamin A supplemented group showed a fourfold increase.

Carotenoids were present in significant amounts in the livers of all newborn calves and varied directly with the carotene content of the maternal prepartum diet.

The addition of one million I.U. of vitamin A daily to the normal ration of pregnant cows resulted in an average total fetal liver storage of 97,177 I.U. of vitamin A.

Evidence has been obtained that the prepartum diet of the normal bovine may influence markedly the vitamin A and carotene reserves of the newborn calf.

P4. Utilizing all Colostrum in Calf Feeding. H. E. Kaeser and T. S. Sutton, The Ohio State University and The Ohio Agricultural Experiment Station.

The importance of colostrum in the nutrition of the calf is well established, yet a large amount of this important calf feed is either wasted or used in such a way that greatest benefits are not obtained from its superior nutritive value. The belief that prolonged or irregular colostrum feeding will result in "scours" also prevails among practical dairymen. Results in an experiment in which the colostrum feeding period was extended to seven days have been reported. This procedure may not find widespread practical use because of the necessity of storing the excess colostrum for later feeding.

It was considered advisable to study some method of using all of the colostrum produced in the feeding of calves without the inconvenience and hazards of colostrum storage. The present paper is a progress report of this investigation.

At birth the calves are assigned to comparable groups and managed similarly. After three days during which each calf receives colostrum from its own dam Group II receives Holstein milk. Any colostrum remaining after feeding the cow's own calf is used to replace an equal weight of Holstein Milk in feeding Group I. The daily allotment of milk or milk and colostrum is determined by the weight of the calf. Hay and grain are provided ad libitum after the third day. Data are obtained on weight and blood plasma vitamin A and carotene.

The summarized data for 33 calves shows that the calves receiving the extra colostrum maintain higher levels of plasma vitamin A and carotene during the first eight weeks. More rapid gains are also observed during the first four weeks. The average gain of the Group I calves being 19.4 lbs. in comparison with 16.2 for the Group II or control calves.

During the first six weeks the extra colostrum fed calves exhibit a vastly superior physical appearance. There is more gloss to their coat, they appear to carry more flesh and are more alert. The amount of colostrum fed is quite variable, ranging from all the daily allowance to none. Abrupt changes from a diet of Holstein milk to part or all colostrum has not created any problems.

P5. The Effect of Vitamin Supplements on Survival of New-Born Calves.* R. G. Hansen, P. H. Phillips, and I. W. Rupel, University of Wisconsin.

The supplementary effects of vitamin A, niacin and biotin fed singly or in combination has been studied with new-born colostrum-free Holstein

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calves. A basal ration composed of commercial skim milk with an added 250 mg. of ascorbic acid was used in all cases. Two levels of vitamin A were used in these experiments, namely 10,000 and 25,000 I.U.

Calves fed the basal ration normally exhibited a looseness of the bowels regardless of the dietary supplements employed. If the looseness reached a severe stage 2–4 grams of sulfathiazole were given for one day and lighter doses thereafter as the condition of the calf indicated. It was apparent that the level of vitamin A fed affected the degree and frequency of the occurrence of these diarrheas. Severe diarrhea occurred in 8 of 9 calves (89%) given 10,000 I.U. while 25 cases occurred out of a possible 36 (69%) when 25,000 I.U. were fed. The occurrence of severe diarrhea was correlated with season. The incidence of severe diarrheas was higher by three fold during the January–May period than it was in the June–December period. Data on 36 calves fed 25,000 I.U. of vitamin A daily indicate a higher survival rate by 25–30% for the summer–fall period. Eight calves of 23 born during the winter period failed to survive on the basal ration while only 1 out of 13 dropped during the summer–fall period failed to survive on the vitamin A niacin fortified basal ration. The contrast was even more marked in a limited number of observations with Guernsey calves. This evidence indicates that factors other than vitamin A and niacin are involved in the survival of the new-born calf. A nutritional factor, or factors present in the skim milk or improved intra-uterine nutrition is postulated.

Plasma vitamin A concentrations determined at birth, 24–36 hours and 6–14 days indicated values of 4 µg., 10 µg. and 13 µg. of vitamin A per 100 ml. respectively for the colostrum-fed calf. All values at birth were found to be about 4 µg. per 100 ml. which confirms previous observations. Four calves given the basal ration plus 10,000 I.U. of vitamin A only showed little increase in plasma A concentration and only 1 survived. Six calves fed a supplement of 10,000 I.U. plus niacin (50 mg.) had 6 µg. and 12 µg. of vitamin A per 100 ml. at the 24–36 hours, and 6–14-day interval respectively and 50 per cent survived. When the vitamin A level was increased to 25,000 I.U., 27 out of 36 calves survived. At this level of vitamin A administration no measurable supplementary effect of niacin, or niacin and biotin, was observed.

From these studies it appears that (1) new-born calves fed the skim milk basal ration require vitamin A in amounts in excess of 10,000 I.U. per calf per day for better than 50 per cent survival; (2) that 25,000 I.U. per day gives a satisfactory survival rate, (3) that a favorable supplementary niacin effect is not observable when adequate levels of vitamin A are ingested, and (4) that some factor, or factors are present either in "summer skim milk" or in intra-uterine life or both which favorably influences survival of young calves dropped between June 1 and January 1.

P6. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage in Calves. J. W. HIBBS AND W. E. KRAUSS, Ohio Agricultural Experiment Station, Wooster, Ohio.

In connection with experiments designed to study the effect of supplementary vitamin feeding on the control of scours, a study was made of the blood plasma vitamin A, carotene, and ascorbic acid, and of total vitamin A liver storage of calves under two different systems of supplementary vitamin feeding.

In the first experiment, in which both Holstein and Jersey calves were used, one-half (15) of the calves received a multivitamin capsule every day for the first 20 days. These capsules contained 300 U.S.P. units of vitamin D, 250 mg. of ascorbic acid, 50 mg. of niacin, and 10,000 U.S.P. units of vitamin A. The other half (15) of the calves received a placebo capsule containing a biologically inactive oil.

Blood plasma vitamin A analyses showed that a peak was reached at about 21 micrograms per 100 ml. on the third day. This was followed by a steady decline until the twenty-first day when the control group averaged 11.1 micrograms per 100 ml. and the experimental group 14.8 micrograms per 100 ml. The higher value in the experimental group is attributed to the supplementary vitamin A fed.

Vitamin A storage, aside from carotene, in the liver of some of the male calves showed that the control group had stored approximately 36,000 U.S.P. units while the experimental group had stored approximately 62,000 U.S.P. units.

No difference between the groups was noted in the levels of plasma carotene and ascorbic acid.

In a second experiment three groups were treated as follows: Group I (10 calves) received no vitamin supplement. Group II (13 calves) received 250,000 units of vitamin A in capsule form on the third and tenth days. Group III (12 calves) received 250,000 units of vitamin A on the third and tenth days, plus 50 mg. of niacin daily for the first 20 days.

Vitamin A, carotene, and ascorbic acid determinations were made on the 3rd, 10th, and 20th days. In addition liver vitamin A storage was determined on the 20th day in the male calves. Blood plasma vitamin A and liver storage analyses indicated no significant difference between Groups II and III. At 10 days Groups II and III averaged 17.2 micrograms per 100 ml. whereas Group I averaged 11.2. At 20 days Groups II and III averaged 15.1 and Group I averaged 10.8. At 20 days total vitamin A liver storage determinations showed that Groups II and III had stored an average of 158,000 U.S.P. units more than Group I.

In these experiments scours occurred just as frequently in the groups receiving supplementary vitamins as in the control groups. Plasma vitamin A and carotene, however, were found to be lower in calves that had scours than in calves that did not have scours. Holstein calves in both experiments showed a higher average level of blood vitamin A and a lower carotene level. Scours were much more prevalent in the Jersey calves.

The falling off of blood vitamin A during the first few weeks can largely be offset by feeding additional vitamin A. It is shown further that regardless of the amount of vitamin A fed the blood level seldom exceeds 25 micrograms and that the blood level is not a good criterion of liver storage except when liver storage is low.

P7. The Relationship of Prepartum Diet to the Carotene and Vitamin A Content of Colostrum.

J. W. THOMAS, A. A. SPIELMAN, AND K. L. TURK, Department of Animal Husbandry, Cornell University.

Four different rations were fed to Holstein and Guernsey cows during the last sixty days of their gestation period.

The rations used and the number of animals in each were: (A) Straw plus a concentrate mixture, nine animals; (B) Fitting ration consisting of mixed hay, corn silage, and concentrate mixture, six animals; (C) Fitting ration plus one million I.U. of carotene in oil, six animals; (D) Fitting ration plus one million I.U. of vitamin A as a fish-liver oil concentrate, seven animals.

All animals were fed the herd ration as soon as they freshened. Samples of the first and sixth milkings were obtained for carotene and vitamin A analysis. With some cows the total colostrum produced during the first three days was collected. This was analyzed and the total output of carotene and vitamin A in the colostrum was obtained.

The vitamin A concentration in the first milking of cows on rations (A), (B), and (C) was approximately the same, averaging 3.5 micrograms per ml., about eighteen times that in normal milk. Group (D) produced colostrum with 6.3 micrograms of vitamin A per ml. The carotene concentration in the first colostrum sample of group (C) was somewhat higher than in the other groups.

In the sixth milking the concentration of vitamin A of group (D) was three times that of the other groups. The carotene concentration of the sixth milking was highest in group (C) while group (A) was noticeably lower than either group (B) or (D).

The results expressed per gram of fat show the same relationship in both the first and the sixth milkings.

The first colostrum sample of one of the vitamin A supplemented cows analyzed 61.5 micrograms of carotene and 886 micrograms of vitamin A per gram of butterfat.

The total vitamin A output in the first three days' colostrum was variable within each group. The averages were 170 mg. for four vitamin A supplemented cows, 53 mg. for nine cows fed the normal fitting ration, and approximately the same for four cows supplemented with carotene. Total carotene output of the carotene supplemented group was double that of groups (B) and (D).

The concentration and total output of vitamin A and carotene in cow's colostrum has been demonstrated to be influenced by the ration fed during the two months immediately prior to parturition.

P8. Vitamin A and Globulin Content of Colostrum Milk.* R. G. HANSEN, P. H. PHILLIPS, J. W. WILLIAMS, AND V. R. SMITH, University of Wisconsin.

The vitamin A content of colostrum milk from 43 cows has been studied with particular reference to the rate of decline, the effect of number of lactations and the effect of milking procedure upon vitamin A concentration of colostrum. Further, the effect of colostrum upon the serum protein fractions of calf blood have been studied.

Colostrum milk samples were obtained from cows which had been completely milked out the first two milkings either with or without the aid of oxytocin. The samples were quickly frozen and stored at −4° C. until analyses could be made.

The results show that in eighteen Holstein heifers fed identical rations that the vitamin A content of the colostrum from the first lactation was more than double the vitamin A content of the colostrum from the second lactation, while the carotene content of the colostrum was about the same for each of the two lactations. With cows that were allowed to remain with the calvés but in addition were milked out "to balance the udder" the chief drop in vitamin A potency occurred during the first three milkings. Thereafter there was a gradual decline to normal concentrations in milk. With cows isolated from the calves the vitamin A potency of their colostrum from the first and second milkings was about the same. However, with cows which were allowed to remain with the calves and were milked out twice daily, there was a decided drop in vitamin A potency between the first and second milking. The intravenous injection of 10 I.U. of oxytocin at the time of milking in cows isolated from their calves caused a decided drop in vitamin A potency from the first to the second milking.

The serum protein fractions of colostrum-fed calves and colostrum-free calves fed skim milk were studied electrophoretically. At birth the gamma globulin component of all calves was extremely low. Within eighteen hours after the ingestion of colostrum the gamma globulin fraction increased to approximately 10 per cent of the serum proteins. In this same period the beta globulin showed a slight increase while the alpha globulin fraction showed a corresponding decrease. The albumin fraction appeared to be unchanged. In calves fed skim milk the gamma globulin fraction had increased only slightly at two weeks time and even at eight weeks had not reached a normal level. In one new-born colostrum-free calf the serum gamma globulin fraction increased significantly when the calf was fed skim milk plus seventy grams of gamma globulin isolated from colostrum. There was no increase in gamma globulin component in a seventeen-day-old calf, which had been fed on skim milk from birth, when seventy grams of gamma globulin isolated from colostrum were given.

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P9. The Effect of Vitamin A Deficiency on Reproduction in Dairy Bulls.

R. E. HODGSON, S. R. HALL, W. J. SWEETMAN, H. G. WISEMAN, AND H. T. CONVERSE, Bureau of Dairy Industry, Agricultural Research Administration, United States Department of Agriculture.

Twelve dairy bulls were made to develop typical vitamin A deficiency symptoms at different ages by adjusting their vitamin A intake. Their breeding abilities and the quality and fertilizing capacities of their semen which they produced while in a deficient condition and in some cases while supplemental carotene was fed after a period of avitaminosis A was studied.

When deficiency symptoms appeared before the expected breeding age, bulls failed to breed; when they appeared at about the breeding age they frequently failed to breed shortly thereafter, but when they appeared after they had started breeding this capacity was maintained. Bulls that would not mount and serve the artificial vagina invariably yielded semen by the massage technique. Seven of the vitamin A-deficient bulls (all that were tested more than twice) were proved fertile by artificially inseminated cows.

Generally the semen produced by vitamin A-deficient bulls was low in concentration, had a high percentage of abnormal sperm, a high pH, and did not store well.

At autopsy the bulls all had cystic pituitary glands; three bulls with badly damaged glands were proved fertile. The epithelium of the seminiferous tubules of the testes of the bulls was found to have undergone degeneration—in some cases extensively.

Four bulls that had lost their ability to breed, when fed 8 to 11 micrograms of supplemental carotene per pound weight daily, started breeding but the quality of the semen was not substantially improved.

P10. Relation of Carotene Levels to Fertility in Dairy Bulls,

I. R. JONES, J. O. SCHNAUTZ, AND J. R. HAAG, Oregon State College.

This study has been continued using the same carotene-deficient ration of molasses, dried beet pulp and a grain mixture supplemented with minerals and vitamin D as first reported at the 1944 annual meeting. Of the five bulls on experiment at present, Nos. 51 and 55 have received alfalfa meal to supply daily approximately 25 micrograms of carotene per kilogram of body weight; and Nos. 52 and 59, 35 micrograms carotene per kilogram of body weight. Bull No. 57 was fed 65 micrograms of carotene per kilogram of body weight until 445 days of age, when the level was reduced to 15 micrograms. Bull No. 60, being fed at the 65 micrograms of carotene level, broke a leg in his stall at 450 days of age and was killed.

The bulls now about 3½ years of age, range in size from about 1250 to 1450 pounds without any particular relation indicated between the carotene level fed and the weight of the animals.

Four of the five bulls have been used in natural service and semen has been collected artificially for examination. One bull, No. 55, has never shown sexual desire and thus has neither service nor examination records. This particular bull has always been quite timid whereas the other bulls, Nos. 51 and 52, that became blind in their early growing period, have shown normal bull behavior.

It has been noted that cows calving became pregnant to a small number of average services. Cows not becoming pregnant were usually bred only once.

Semen samples have usually shown excellent original motility and good duration of motility in stored samples in the case of Nos. 57, 52, and 59. Samples from No. 51 have been quite variable in original motility but the duration of motility has been good. Bull. No. 59 has at times been slow on service and twice has refused service.

It appears from the results to date that carotene levels in the range of 15 to 35 micrograms per kilogram of body weight are sufficient to allow Jersey bulls to retain their fertility.

HORMONES, BLOOD, AND DISEASE

P11. The Adrenal Cortex in Relation to Ketosis in Dairy Cows. J. C. Shaw, University of Maryland.

The subcutaneous administration of from 100 to 150 ml. of adrenal cortex extract (Wilson) per day resulted in marked improvement within 24 hours in both the symptoms and the blood picture in each of the four cases studied. A number of tests were conducted to determine whether an adrenal insufficiency is involved in the development of ketosis in cows. The following favor the view that such an insufficiency is involved: (1) Ketosis in cows is a hypoglycemic condition, (2) the symptoms are often apparent at glucose levels which do not produce symptoms in normal cows, (3) cortical extract has elicited a favorable response in each of four cases studied, (4) cows with ketosis are more sensitive to insulin injections than normal cows, and (5) the blood glucose response to adrenalin injections is slight. The following are not in accord with the view that an adrenal insufficiency is involved in the development of ketosis in dairy cows: (a) the plasma sodium and potassium are normal, (b) the intravenous glucose tolerance curve is not typical of that observed in cortical insufficiency and (c) cows with mild cases of ketosis usually recover following a single intravenous injection of glucose. While some of the data cannot be properly evaluated until information is obtained on adrenalectomized cattle, it is believed that the data obtained so far favor the view that ketosis in cattle is not due to an adrenal insufficiency. Since extracts of the adrenal cortex promote glyconeogenesis even in normal animals, this material may have a beneficial effect even though an adrenal insufficiency is not involved.

P12. Effects of Stilbestrol on Lactation and Reproduction.

SIDNEY P. MARSHALL,* R. B. BECKER, AND P. T. DIX ARNOLD, Florida Agricultural Experiment Station, Gainesville, Fla.

Diethylstilbestrol, or stilbestrol, was synthesized in England in 1938, and in 1940 was found to stimulate udder development and lactation in goats. An entire issue of Journal of Endocrinology (Jan., 1944) reported investigations in England with cows and virgin heifers. The compound was used on heifers, cows, and goats at the Minnesota, Missouri, Kentucky, and Louisiana stations, usually causing rapid development of the udder with subsequent milk secretion. It appeared to depress milk production with animals already in lactation.

Methods

At the Florida station, 14 heifers and 5 dry cows were injected subcutaneously with stilbestrol dispersed in "Wesson" cottonseed oil (10 mgm. per ml.). Each animal received 1 milliliter daily for 5 days, then 3 injections weekly of 2 milliliters until lactation began. With some, the injections were continued for 32 to 89 days longer.

Sexual excitation

Early evidence of reaction to stilbestrol was marked sexual excitation, extending over 2 to 4 days in 16 out of 87 instances observed. Injections were discontinued at initiation of lactation with 10 animals, 4 of which showed excitation later a total of 6 times. Excitation continued longer among the 8 heifers and 1 cow with the injections continued into lactation. More generally, a quiescent period followed discontinuance of injections.

Udder development and milk yields

Responses of udder development to stilbestrol varied widely. Lactation began in the heifers in 14 to 64 days. One cow lost flesh; did not come into milk, and was discontinued ofter 71 days. Between 13 and 53 milligrams of stilbestrol were administered per animal to time of lactation, with a total of 71 to 93 milligrams in the heifers with injections continued into lactation.

The animals were milked by machine twice daily after fullness of the teats indicated that milk secretion had begun. Secretion was at a lower level, and the peak of lactation appeared to be attained more slowly than with 17 related animals following normal calving. The average peak of production over 10-day periods ranged between 13 and 24 pounds of milk daily, tending to be lower among the animals with which injections were discontinued at first milking. Milk yields in 300 days ranged between 2,838 and 4,151 pounds each for the heifers. One cow completed 300 days with a yield of 4,080 pounds of milk. Seven heifers milked from 400 to 644 days. Continuation of lactation long after injections ceased may be explainable as maintained primarily by mechanical stimulation.

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* Now at Clemson Agricultural College, Clemson, S. Car.

Reproduction

The initial sexual excitation was independent of ovulation. Intentionally, no animal was bred during the first 90 days in lactation. Discontinuance of stilbestrol injections was followed by sexual quiescence. Later, 6 animals were bred on apparent estrus a total of 7 times, resulting in a single conception. Since most animals in this investigation were irregular breeders when selected, the low proportion of conception cannot be attributed to the stilbestrol.

P13. Elimination of Male Hormone by Lactating Dairy Cows. C. W. Turner, Missouri Agricultural Experiment Station.

Riley and Hammond (1942) presented the first evidence that dairy cattle excreted appreciable amounts of androgenic hormones in the feces. When baby chicks were fed dried cow manure as an ingredient of the starter ration, the chicks' combs developed an intense red color and grew in size precociously.

This observation was of interest to us from several points of view. If true, dried cow manure would serve as a cheap source of androgenic hormone for feeding experiments. Second, it would be of interest to determine the factors influencing the rate of male hormone elimination in male and female cattle. Third, it would enable one to determine the normal role of the male hormone, if any, in the growth of the udder, and the secretion and maintenance of milk production.

While our observations are still quite limited, I have confirmed the report of the elimination of relatively large amounts of androgenic hormone in the feces of lactating dairy cows. The cow manure assayed showed the presence of androgens in one kilo equal orally to the effect produced by the feeding of 200 mg. of methyl testosterone to chicks during a 4-week period. The manure from bulls contained much less. Manure from non-lactating sheep and goats also contained less male hormone than from lactating cows.

While a few reports on the stimulating effect of androgens upon the growth of the mammary gland and the lactogenic hormone have been made, the observation of the normal elimination of large amounts of androgens by lactating dairy cows will stimulate renewed interest in the normal role of this hormone in lactation.

P14. A Technique for Intravenous Feeding of Ruminants with Some Observations on the Effect of the Administration of Various Substances upon the Secretion of Milk Fat. A. I. MANN, University of Connecticut and J. C. SHAW, University of Maryland.

Various substances were fed as the sole source of nutriment to lactating goats or cows via the abomasum by means of a rubber catheter and by intravenous administration by the technique described below. A No. 10 rubber catheter was fixed in the subcutaneous abdominal mammary vein. The catheter was attached to a constant drip bottle by means of rubber tubing. By placing a pulley and a sliding weight between the drip bottle and the catheter, the animal was able to lie down at will. Triacetin and glucose fed at a constant rate intra-abomasally for 19 and 41 hours respectively did not prevent the decrease in lower fatty acids and increase in unsaturation of milk fat typical of fasting. Constant intravenous feeding of glucose, protein hydrolysates, sodium acetate and sodium oleate likewise did not prevent these changes. As much as 1985 gm. of glucose was injected during a 47-hour period, 1984 gm. of sodium acetate during a 22-hour period, and 138 gm. of oleic acid during a 15½-hour period. In the latter case, the cow was fasted for approximately 20 hours before the oleic acid was administered. The Polenske and Reichert-Meissl values obtained indicate that none of the above materials serve as a precursor of the lower acids of milk fat. Various fat emulsions were prepared. Purified phosphatides were used in one preparation as a stabilizer. In another, a monoglyceride and bile salts were used. These preparations were homogenized at 3,000 lbs. pressure. Emulsions were prepared in which the average diameter of the fat globule was approximately 1 micron. The intravenous injection of these fat emulsions in quantities as low as 10 ml. produced immediate and severe anoxia.

P15. Blood Studies of Louisiana Dairy Cows. II. Calcium, Inorganic Phosphorus, Hemoglobin Value, Erythrocyte Count, Leucocyte Count, and Percentages of Types of Leucocytes. L. L. RUSOFF AND P. L. PIERCY,* Louisiana Agricultural Experiment Station.

Variations in the blood picture of high-producing dairy animals in two separate herds which were fed and managed better than average is presented. The blood picture of low producing animals of an experimental herd which were on a basal diet containing poor quality hay as the only source of roughage, supplemented with either Ca or P, or both Ca and P, or no minerals, is included.

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* Dairy Research and Veterinary Medicine Departments, respectively.

Analysis were made on pooled citrated blood drawn on three consecutive days from 38–46 animals every month or every two months over a period of approximately 2 years.

A statistical analysis of 753 determinations for Ca and P, and 613 observations for each of the other constituents was made. Using the analysis of variance it was found that significant differences existed between animals in a herd and between months (within animals) for calcium and phosphorus values. There were significant differences between months (within animals) in a herd for hemoglobin values, and between animals for the leucocyte count. No significant seasonal variations were found. Only low and non-significant correlations were found between either Ca or P and the hemoglobin value, erythrocyte, or leucocyte counts. Significant differences were found between herds for hemoglobin content, erythrocyte count, leucocyte count, percentages of lymphocytes, neutrophiles and monocytes.

Poor quality hay in the ration of the experimental herd did not seem to influence the blood calcium content or amounts of the various cellular constituents, but the phosphorus and hemoglobin values were significantly different from those of the other herds.

The range of means and their standard errors for the herds are as follows:

Calcium, 10.89 ± 0.21 to 11.36 ± 0.15 mg. % plasma.
Phosphorus, 5.02 ± 0.07 to 5.77 ± 0.13 mg. % plasma.
Hemoglobin, 11.19 ± 0.27 to 13.39 ± 0.22 gm. per 100 cc. blood.
Erythrocytes, 4.89 ± 0.030 to 5.72 ± 0.035 millions per cmm. blood.
Leucocytes, 8,411 ± 0.31 to 10,268 ± 0.45 per cmm. blood.
Lymphocytes, 54.46 ± 0.86 to 57.26 ± 0.79%.
Neutrophiles, 29.35 ± 0.65 to 32.64 ± 0.87%.
Eosinophiles, 6.40 ± 0.38 to 7.32 ± 0.37%.
Monocytes, 5.49 ± 0.06 to 6.38 ± 0.04%.
Basophiles, 0.37 ± 0.09 to 0.58 ± 0.08%.

P16. Studies of the Growth and Blood Composition of Dairy Calves Fed Remade Skimmed Milk after Three Days of Age. P. M. REAVES AND L. R. ARRINGTON, Virginia Polytechnic Institute.

Calves were removed from their dams on the third day after birth and placed on an experimental ration of remade skimmed milk supplemented with vitamins A and D in the form of carotene and viosterol or cod-liver oil. Grain and hay were fed as soon as the calves would eat it.

Calves of the Holstein breed were raised to practically normal size at four months of age by this method. The results with Jersey calves were only partially successful. There was no difference in the calves receiving the different vitamin supplements.

The fat content of the blood of the calves fed remade skimmed milk was lower than that of a control calf fed whole milk. When the calves on skimmed milk consumed considerable quantities of hay and grain the blood fat rose to a level as high as that of the control calf on whole milk.

The blood carotene was higher in the calves receiving the carotene supplements than that of the control calf on whole milk or that of the experimental calves receiving cod-liver oil as a supplement.

The vitamin A content of the blood was higher in calves fed cod-liver oil as a supplement than in the case of the control calf or of the calves fed the carotene concentrate.

P17. The Effect of Complete Milking on Blood Serum Calcium of Cows at Parturition. VEARL R. SMITH, University of Wisconsin.

This study was undertaken to ascertain the effect of complete milking shortly after calving on the blood serum calcium. The cows were of Holstein, Jersey, and Guernsey breeds. The cows were divided into two groups by alternating at freshening within a breed.

The cows in the control group were managed conventionally by permitting the calves to remain with the cows for three days after calving. The cows in this group were partially milked twice daily beginning the day subsequent to calving.

The calves were removed from the dams of the experimental group before nursing, and the cows were milked completely with the aid of an injection of 10 I.U. of oxytocin, usually within an hour of calving. Oxytocin injections were made at each milking for the first five days subsequent to calving.

Samples of venous blood were drawn daily from five days prior to the anticipated day of parturition, and daily to five days subsequent. The samples were drawn within an hour of the same time daily. Calcium was determined by a modification of the Clark-Collip method.

Calcium determinations have been made on 29 cows: fourteen in the control and fifteen in the experimental group. Five of the cows in the control group have been treated for milk fever, and two in the experimental group. Production data indicate that the cows in the experimental group attained peak production sooner than the cows of the control group.

P18. The Occurrence of Various Bacterial Infections and Their Significance in Bovine Mastitis. L. A. BURKEY, W. W. SWETT, AND CECILIA R. BUCKNER, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Although the results for mastitis investigations reported elsewhere, and in the past by the Bureau of Dairy Industry, have shown that *Streptococcus agalactiae* is the predominant infective organism associated with bovine mastitis, detailed studies by this bureau during the last 18 months have shown that bacteria other than *S. agalactiae* are the most frequent cause of mastitis infections.

The results of this study for 1945 show that the percentage of quarters infected with *S. agalactiae* was 6.3, with *S. dysgalactiae* 10.4, with strains of *S. uberis* 25.5, with strains of *S. viridans* 19.1, with strains of *S. fecalis* 10.6, with staphylococci 23.2, and with pseudomonades 4.9. The streptococcal infections during 1945 were 71.9 per cent of the total infections.

The percentages on the same basis for the first 4 months of 1946 were as follows: *S. agalactiae* 0.9, *S. dysgalactiae* 6.1, *S. uberis* 34.0, *S. viridans* 14.0, *S. fecalis* 5.0, staphylococci 28.5, and pseudomonades 11.5. During this period in 1946, the streptococcal infections were 60 per cent of the total infections.

A definite explanation for the recent decrease of *S. agalactiae* infections in bovine mastitis and the predominance of other bacterial infections, such as *S. uberis*, *S. viridans*, and staphylococci, and the continued trend in this direction has not been determined. These results have been obtained in conjunction with studies in which all infected quarters have been treated with chemo-therapeutic agents. The results of treatment for 1945 show that *S. dysgalactiae* and *S. agalactiae* are the most susceptible to these treatments, while *S. uberis*, pseudomonades, *S. fecalis*, *S. viridans*, and staphylococci are definitely less susceptible to the treatments, in the order given.

These results suggest that the greater resistance to treatment by chemotherapy may be one factor responsible for the increased predominance of these infections in a particular herd.

The predominance of *S. uberis* and staphylococci and the continued prevalence of pseudomonades in the herds studied are significant, not only because these organisms are relatively resistant to treatment but also because the infections produced are in most cases equally as serious as *S. agalactiae* infections.

These observations suggest that the future approach to the control of mastitis infections may have to be broadened to adequately provide for the health of the dairy herd.

P19. Some Results in Treating Mastitis by Means of Sulfanilamide, Tyrothricin, and Penicillin. H. A. HERMAN AND O. S. CRISLER,* University of Missouri.

Several of the more recently suggested treatments for mastitis in dairy cows have been used over a 3-year period in the University of Missouri dairy herd.

Sixty-three cows in the Station dairy herd showing streptococci infection, and occasionally staphylococci in one or more quarters were treated by one or combinations of the following udder injections: Colloidal silver oxide (Novoxil), sulfanilamide and mineral oil (Sulvetil), sulfanilamide-iodine-mineral oil, tyrothricin, and more recently penicillin. Diagnostic tests consisted of the Hotis test, microscopic examination of milk, leucotyte count, strip cup, and physical examination of the udder. The infected cows were treated at regular intervals and an examination of the milk made at 2-week intervals following treatment and until the animal maintained a clean test for 12 weeks.

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* Department of Dairy Husbandry and Department Veterinary Science cooperating.

Thirty-four cows (94 quarters) were treated with an emulsion of mineral oil, sulfanilamide (30–35%) and iodine, 1 part to 2000 parts oil. Treatment consisted of daily injections for four days of 50 to 60 cc. of the sulfanilamide, iodine and mineral oil per quarter. Seventy-five to 80 per cent of the quarters were free of streptococci 2 to 4 weeks after treatment and the majority have remained clean. Penicillin has been found to be about 80 per cent effective in treating mastitis infections in 13 cows involving 33 quarters. Treatment consisted of injecting the infected quarters with 100,000 to 200,000 units of penicillin in saline solution, in 2 to 8 doses ranging from 25,000 to 50,000 units per dose.

No swelling or inflammation following the use of penicillin has been noted.

Four cows (11 quarters) were treated with Sulvetil and all but one animal (2 quarters) have become negative.

Twelve cows have been treated with tyrothricin and following one to five treatments all but two have become negative.

Two cows were treated with Novoxil during the dry period. One freshened free of streptococci in the udder and the other was positive.

Sulfanilamide injections produced no swelling or inflammation in the udder. Novoxil treatments resulted in severe swelling and inflammation the first 2 to 5 days following its use.

Tyrothricin produced from mild to fairly pronounced inflammation which persisted from 12 to 40 hours.

P20. The Effect of Ingestion of DDT upon Dairy Cows.

N. N. Allen, H. A. Lardy, and H. F. Wilson, University of Wisconsin.

Growers of canning peas have found DDT effective for controlling aphids. The green vines, stored as stack silage, are fed to dairy cows and to meat animals during the winter months, and the question arises as to the possible toxic effect of DDT residue in this silage upon the health of the animals and upon the consumers of their milk and meat.

Pea vines were stored at harvest time in two silos. DDT was added at the rate of 1 pound per ton to the vines in one silo, while the other lot, serving as a control, was ensiled without DDT.

Beginning November 9, the silage was fed to dairy cows at the rate of

3 pounds per 100 pounds live weight daily, with clover hay of fair quality and a 16 per cent protein concentrate. One group of 4 Holsteins and 1 Guernsey received the treated silage, while a similar group was fed the untreated silage. Blood and milk samples were taken frequently during the course of the experiment. Milk from the two lots of cows was fed to rats to test for toxicity.

Three cows received the treated silage for 118 days, one for 141 days, and the fifth was slaughtered after 127 days on the treated silage. One of these dropped a heifer calf after 67 days of silage feeding. This calf received milk from this group of cows for 75 days. A second cow gave birth to a bull calf after 102 days of silage feeding. This calf was slaughtered after 33 days on the milk from the cows receiving treated silage. A third calf, born to a control cow, was given milk from the cows getting the treated silage after the colostrum period to 33 days of age.

None of the animals showed any evidence of toxic effects from the DDT of the silage. The two animals slaughtered showed no gross symptoms in the body organs. The calcium, carotene, vitamin A, ascorbic acid and hemoglobin levels of the blood were not affected. Rats fed milk from the cows receiving the treated silage as the sole diet grew as well as those receiving milk from the control cows, and showed no symptoms of DDT poisoning.

The milk from the cows receiving the treated silage contained 15.6 parts per million of DDT near the close of the experimental feeding period. Analyses of the tissue of the slaughtered animals indicated 3.8 parts per million in the muscle tissue, 6.1 parts per million in the liver, and 221 parts per million in the depot fat of the cow, while the calf had 3.1, 6.2 and 305 parts per million respectively for the corresponding tissues.

One Jersey cow was fed 24 gm. of DDT daily mixed with her feed for a period of 157 days without showing any symptoms of toxicosis. Her milk contained 44 parts per million of DDT and was sufficiently toxic to inhibit growth of rats. Her depot fat, sampled by biotomy one month after discontinuing DDT feeding, contained 380 parts per million of DDT, and her milk 7 parts per million, indicating storage and residual effect. It should be emphasized that this cow received much larger amounts of DDT than would be probable under practical conditions.

A small lot of silage was available from a field-dusted experimental field. This was found to contain less than 1 part per million of DDT when fed, and no DDT was found in the milk of the cows to which it was fed.

THYROPROTEIN, PROTEIN AND MINERALS

P21. Thyroid Secretion Rate of Growing, Pregnant, and Lactating Rats.

R. A. MONROE AND C. W. TURNER, Missouri Agricultural Experiment Station.

In this study, a quantitative determination has been made of the thyroid secretion rate of growing male and female rats (fifty to three hundred grams) and of pregnant and lactating rats. The rate of thyroid secretion was measured by the technique suggested by Dempsey and Astwood in 1943. The principle of the method, in brief, is this: The amount of thyroxine is determined which, by daily injection, will prevent thyroid hypertrophy in thiouracil-treated rats. This amount is considered to be equivalent to the amount of thyroid hormone the rat would produce normally. The daily thyroid secretion rate in all groups in this study, therefore, is expressed in terms of micrograms of d,l-thyroxine per hundred grams of body weight.

The thyroid secretion rate of the growing male rats showed no significant change throughout the body weight range; the average daily rate of secretion in all groups was approximately 3.5 micrograms per hundred grams of body weight. The growing females, on the other hand, showed a steady decline in thyroid secretion rate as their body weight increased. The most marked decline was noticed between the range of fifty to two hundred grams body weight, when the secretion rate of the thyroids dropped from over 4.5 micrograms to slightly over 3.0 micrograms per hundred grams body weight. From this point on, the rate of decline was slower. In the two hundred and fifty to three hundred gram weight group (ave. 266.2 gm.), the thyroid secretion rate had declined to 2.82 micrograms per hundred grams body weight.

The data concerning the thyroid secretion rate of pregnant rats show no significant variation from that of normal female rats of the same body weight. Likewise, lactating rats also seem to have a thyroid secretion rate which does not vary from normal.

In addition to the data presented above, we have figures which seem to prove conclusively that, while thiouracil does pass through the mammary gland in considerable quantities, thyroxine does not pass through into the milk, even in doses above the physiologic level. Nor is the growth rate of the young rats affected, in the first fifteen days at least, by the administration of thiouracil and/or thyroxine to the mothers.

P22. Some Physiological Effects of Feeding Thyroprotein to Dairy Cows.

L. A. MOORE, Division of Nutrition and Physiology, Bureau, of Dairy Industry, Agricultural Research Administration, United States Department of Agriculture.

Some rather significant physiological observations have been made in an experiment where thyroprotein is being fed to milking cows for periods as long as eight months. When T.D.N. was fed according to the Morrison standard marked losses in body weight were noted. After considerable loss in body weight, milk production declined at a fairly rapid rate. The loss in body weight and decline in milk production could be checked by increasing the T.D.N. intake by 25 per cent above the Morrison standard.

It has further been noted that after cows have been receiving thyroprotein for four to eight weeks and there has been considerable loss in body weight and milk production has declined sharply, the heart rate will also decline. When the feed intake is then increased 25 per cent the heart rate is also increased. It would appear that the heart rate is governed by the amount of energy the body has available, whether from the body tissues or from feed or both. These observations must be considered preliminary and further data on these points are being gathered.

P23. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over an Extended Period of Time.

RALPH P. REECE, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Nine dairy cows received daily either 10 or 15 grams of thyroprotein in their grain ration for periods of time varying from 3 months to 17 months. Three of the 4 cows initially fed 10 grams of thyroprotein showed an increase in milk production and 4 of 5 cows initially fed 15 grams of thyroprotein showed increases in milk production. Soon after the feeding trial started 8 of the 9 cows secreted milk with a higher fat content than might have been expected otherwise. For the entire length of the feeding period 6 of the 9 cows secreted milk with a higher fat content than that secreted in a similar segment of either a previous or a subsequent lactation period. Six of the 9 cows produced more milk and 3 produced less milk during the thyroprotein feeding period than that secreted in a similar segment of either a previous or a subsequent lactation period. Of the three cows not showing an increase in milk production during the entire thyroprotein feeding period only one failed to show an initial response in milk production.

P24. Effect of Feeding Iodinated Casein to Dairy Cows on the Protein Composition and Content of Milk.

A. H. VANLANDINGHAM, GEORGE HYATT, JR., AND CHAS. E. WEAKLEY, JR., West Virginia Agricultural Experiment Station, Morgantown, West Virginia.

Previous data obtained at this Station and elsewhere show clearly that active iodinated casein (thyroprotein) when fed to dairy cows caused an increase in the fat content of the milk. Only relatively small changes in the solid-not-fat have been observed.

During the winter and spring of 1946 iodinated casein supplied by the Cerophyl Laboratories, Kansas City, Missouri, was fed to a group of three Jerseys and four Holstein cows to study the effect of iodinated casein on the protein content and composition of milk. Changes in the composition of milk proteins are of particular interest because of the relationship of such changes to the development of mastitis. The Jerseys were fed 10 to 15 grams and Holsteins 15 to 20 grams daily. Feeding was begun from two and one-half to three months after calving.

Composite samples of milk collected one day each week from individual cows fed iodinated casein and from a similar group of control cows not receiving iodinated casein were analyzed for total nitrogen by the Kjeldahl method. Casein was precipitated and nitrogen determined in the filtrate as non-casein nitrogen as described by Rowland (J. Dairy Res., 9: 42, 1938). Fat was determined on composite samples twice each week. Changes in heart beat, respiration rate, body weight, and body temperature were observed weekly.

Results obtained show clearly that the feeding of iodinated casein resulted in an increase in the rate of heart beat, respiration rate, milk and fat production and per cent of fat in milk.

Results also show no appreciable change in the total nitrogen, casein nitrogen or non-casein nitrogen in the milk due to the feeding of iodinated casein.

However, definite increase in the total nitrogen, non-casein nitrogen and the proportion of non-casein nitrogen to the total nitrogen in milk was observed in cases of clinical mastitis. Clinical mastitis was observed among cows in the herd which did not receive iodinated casein as well as some of those which received iodinated casein.

P25. The Influence of Feeding Synthetic Thyroprotein on Fertility of Bulls.

A. B. SCHULTZE AND H. P. DAVIS, University of Nebraska, Lincoln, Nebraska.

As a preliminary experiment to determine the effect of feeding thyroprotein on fertility, seven bulls including two Jersey, one Milking Shorthorn, one Guernsey and three Holstein, were fed from 1.00 to 1.24 grams of thyroprotein per 100 pounds body weight for a period of thirty days. Observations on initial motility, maintenance of motility during storage at 40° F., and the percentage of abnormal sperm were made. The relative fertility of the semen samples was evaluated by determining the percentage of conceptions resulting on the first and second inseminations in artificial breeding associations. Quality of semen and conception rates were determined for 30 semen samples from the seven bulls prior to feeding thyroprotein and for 27 samples during and 10 days after the feeding of thyroprotein for 30 days. The 30 semen samples collected prior to the feeding of thyroprotein were used in 604 inseminations. The 27 semen samples obtained during and 10 days after the feeding period were used in 586 inseminations.

The initial motility of the 30 samples from the seven bulls prior to thyroprotein feeding averaged 67% compared to 72% during the time thyroprotein was fed. Motility was maintained at an average of 28% at 5 days storage at 40° F. for samples collected prior to the feeding period compared to 40% at 5 days storage for the samples collected during the time thyroprotein was fed.

There was little average change between the percentage of abnormal sperm before and the percentage during the feeding of thyroprotein except in the case of one bull whose semen contained a high percentage (45%) of abnormal sperm before the feeding period. During the time thyroprotein was fed, the percentage of abnormal sperm declined to 20% in this case. However, this particular bull's fertility was not influenced to any significant degree as judged by conception percentages obtained in the field.

With respect to the relative fertility (conception rate) of semen samples collected before and during the feeding of thyroprotein, of the seven bulls used in the experiment, there were 5 whose fertility was increased by feeding thyroprotein. The fertility of the other two bulls was not affected.

With respect to the 5 bulls whose fertility was increased, there was an increase from an average conception percentage of 51.7 before feeding thyroprotein to an average of 55.7 per cent during the 30-day feeding period. A more detailed analysis of the results with these 5 bulls showed that in every case where a semen sample was taken within 7 to 10 days after the beginning of feeding thyroprotein, a lowered conception rate (38% average for 4 samples) resulted. Following this first 10-day period or from 10 to 30 days after the beginning of feeding thyroprotein, there was an increase in fertility. Nineteen semen samples from the 5 bulls showed an average of 58.6 per cent conception rate during this 10–30-day period or a 6.9% increase in conception percentage over the rate obtained during the period prior to feeding thyroprotein. Statistical analysis of these differences showed them to be significant.

Four semen samples taken within 10 days after the feeding of thyroprotein was stopped resulted in increasing the conception rate to 60.4% compared to the 58.6% obtained during the last 20 days of the feeding period.

As a control against any influence that season may have had on fertility, the breeding efficiency of 7 bulls not fed thyroprotein at any time was determined. There was no change in the breeding efficiency during the time of the experiment with these control animals.

P26. Utilization of Non-Protein Nitrogen by Dairy Heifers. I. R. Jones and J. R. Haag, Oregon State College.

This is a progress report of studies begun in 1944 to determine the possible relationship of the sulfur content of feedstuffs to the utilization of non-protein nitrogen by dairy animals. To date, records are available on 32 heifers ranging in age at the start of the feeding period from 150 to 494 days. The heifers, all purebred, have included 18 Holsteins, 8 Jerseys, and 6 Ayrshires, and were paired into two groups on the basis of breed, age, and weight.

The first group of three pairs was fed for 172 days, the second group of 2 pairs for 86 days, and the third group of 11 pairs for 133 days.

The first group was fed bleached oats and vetch hay containing 4.07% crude protein and 0.065% sulfur and a grain mixture with 60% oats, 30% wheat, 7% urea, 2% bonemeal, 1% iodized salt, and 0.2% irradiated yeast. This mixture contained 21.8% crude protein and 0.129% sulfur. The grain mix fed to one of each pair was supplemented with 1% sodium sulfate. The grain mixture with 7% urea was rather unpalatable at the three-pound level fed daily, hence the amount of urea was reduced to 3% of the grain mixture and 5% of peanut meal was added. The second group of calves received the 3% urea grain mix and hay from the same lot. Each calf received 2 or 3 cc. of a 10,000-unit vitamin A oil daily.

The average daily gains for the first group of animals with sulfur supplement were 1.73, 1.61, and 1.83 pounds and for their paired mates without sulfur, 1.63, 1.39, and 1.12 pounds, indicating good utilization of the nitrogen in urea, inasmuch as the hay and grain mixture without urea supplied only 6.2% crude protein.

The average daily gains of the second group of heifers were 1.97 and 1.60 for the sulfur pair and 1.90 and 1.71 for their mates.

The third group of heifers were fed oats and vetch hay containing 6.96% crude protein and 0.066% sulfur. The grain mix contained 60% oats, 17% barley, 10% alfalfa meal, 5% linseed meal, 3% urea,, 2% each of bonemeal and iodized salt, 1% irradiated yeast, and analyzed 18.5% crude protein equivalent and 0.13% sulfur. As previously, 1% sodium sulfate was added to the grain mixture for one of each pair of calves. As consumed by the individual calves, the ration supplied approximately 10% crude protein of which 2.4% was derived from urea.

The average daily gain for 7 Holstein heifers fed the sulfur ration was 1.63 pounds daily and for their purebred mates, 1.58 pounds; for 4 Jerseys fed sulfur 1.09, and their mates 0.90 pounds. The growth of the calves has been on the order of 20% to 30% below normal for their age and breed and, while urea utilization is indicated and 8 of 11 pairs of heifers favored the sulfur supplement, further proof is needed before conclusions can be drawn.

P27. Shark Meal as a Protein Supplement in Dairy Calf Rations.

SIDNEY P. MARSHALL,* R. B. BECKER, AND G. K. DAVIS, Fla. Agr. Expt. Sta., Gainesville, Fla.

Shark meal is a relatively new protein supplement produced by reducing shark carcasses after the liver, hide, fins, and sometimes fillets, teeth, and jaws are removed. The output of shark meal manufactured in Florida and along the Pacific seaboard has increased to considerable proportions during the past 6 years.

Analyses of 19 samples of shark meal prepared by a wet-process method averaged 78.07 per cent of crude protein. Seventeen samples averaged 1.75

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* Now at Clemson Agr. College, Clemson, S. C.

per cent of urea and 0.49 per cent of nitrogen of a non-protein fraction other than urea. Non-protein nitrogen in these forms is equivalent to 8.17 per cent of crude protein.

It is generally agreed that carnivorous and omnivorous animals are unable to utilize some forms of non-protein nitrogen. The ability of ruminants to utilize some non-protein nitrogen indicates that cattle should be able to use the total nitrogen of shark meal efficiently.

To evaluate shark meal as a protein source for young dairy calves, it was compared with roller process powdered skim milk in the rations of Jersey females from 8 through 24 weeks of age. To basal rations composed of ground prairie hay, citrus molasses, ground yellow corn, ground oats, and a mineral mixture, the necessary amounts of shark meal or of powdered skim milk were added to bring the crude protein level to 14 per cent. Corn starch was added to the shark meal rations to adjust the quantity of nitrogen contributed by this protein supplement per pound of total feed to equal that per pound of total feed supplied by powdered skim milk to the other rations. The experiment was divided into 4 periods of 4 weeks each. The rations fed contained 35 per cent of ground prairie hay during the first period, 40 per cent during the second, 45 per cent during the third, and 50 per cent during the fourth. Thirty milliliters of shark liver oil containing approximately 9,000 International units of vitamin A per gram were fed each calf weekly. Feed consumption was equalized between each of the 6 pairs of calves by using the Paired Feeding Technique.

Both of the rations were palatable and the quantities of feed offered were governed with equal frequency by calves on the different rations. The animals fed shark meal rations made an average gain of 118.4 pounds in body weight and 16.3 centimeters in height at withers. The pair mates, fed powdered skim milk rations, gained 117.1 pounds and increased 15.9 centimeters in height. This difference in gain in body weight and increase in height at withers was not significant at the 5 per cent level (Snedecor, 1938). The average rate of growth was slightly below the normal for Jersey females reported by Ragsdale (Mo. Agr. Expt. Sta. Bul. 336, 1934).

P28. Ground Mungbeans as a Protein Supplement in Rations for Dairy Cows. A. H. KUHLMAN, W. D. GALLUP, AND H. W. CAVE, Oklahoma A. and M. College.

Several varieties of the mungbean (*Phaseolus aureus*) have been grown in Oklahoma to a limited extent for about twenty years. During most of this period this annual legume was used as a soil builder and as a hay and silage crop. When importation of mungbeans from China and other parts of Asia for food purposes was interrupted during the recent war, Oklahoma farmers became interested in growing green mungbeans on an extensive scale for sprouting purposes. In 1942, Oklahoma farmers planted 15,000 acres in mungbeans; in 1943, 45,000 acres; and in 1945, 169,000 acres of which 110,000 acres were harvested for seed. This crop yielded 24,200,000 pounds of seed. Even when most of the seed is suitable for food purposes, considerable amounts of cracked and cull beans are usually available for feeding purposes.

Since mungbeans usually contain about 23 to 25 per cent total protein, 2 to 3 per cent oil and 56 to 60 per cent N-free extract, it seemed probable that they might be used to replace a part of the cottonseed meal ordinarily fed in dairy rations.

Two 90-day feeding trials, each consisting of three 30-day periods conducted on the double reversal plan and including a total of 25 cows, have been completed to determine the value of ground mungbeans as a protein supplement in dairy rations. Prairie hay of good quality was used as the sole roughage in each trial. It was fed in such amounts to each cow that the total digestible nutrients furnished by the hay were equivalent to her daily maintenance requirements. Concentrates were fed according to the Morrison Feeding Standard for milk production with adjustments made at seven- to ten-day intervals on the basis of the daily weight records of each cow to secure slight but similar gains on the rations used.

The control concentrate mixture consisted of 350 pounds ground No. 2 yellow corn, 200 pounds ground oats, 200 pounds wheat bran, 250 pounds cottonseed meal (41 per cent protein) and 10 pounds each of salt, ground limestone and bonemeal. In the experimental concentrate mixture, 300 pounds of ground mungbeans replaced 150 pounds of ground corn and 150 pounds of cottonseed meal in the control mixture. Each mixture contained slightly less than 19 per cent protein.

In each of the two trials which have been completed, almost identical results were obtained from the control and the experimental rations. Since 300 pounds of mungbeans replaced 150 pounds of corn and 150 pounds of cottonseed meal, 100 pounds of mungbeans were equivalent to 50 pounds of corn and 50 pounds of cottonseed meal in feeding value. Mungbeans replaced 60 per cent of the cottonseed meal and almost 43 per cent of the corn used in the control mixture.

Very satisfactory seed crops of 12 to 15 bushels per acre are often obtained when mungbeans are planted in June or early July following a small grain crop. Mungbeans, therefore, can be grown as an emergency crop and used as a source of protein in dairy rations.

P29. A Progress Report of a Study of the Effects of Manganese on Calcium Metabolism in Lactating Dairy Cows. J. T. Reid, K. O. Pfau, R. L. Salsbury, and C. B. Bender, Rutgers University.

Three weekly balance trials were conducted at regular intervals with each of 8 Holstein and 4 Guernsey cows during the first 150 days of lactation. The 12 animals comprised 3 collection groups and 4 feed groups. The feed groups received the following: Group I, basal ration (consisting of ground corn, 30 parts; rolled oats, 30 parts; linseed oil meal, 28 parts; wheat bran, 11 parts and plain salt, 1 part); Group II, basal ration + $CaCO_3$, (C.P.) reagents; Group III, basal ration + $CaCO_3$ and $MnSO_4$, (both C.P. reagents); Group IV, basal ration + Mico.* All animals received corn silage and timothy-clover hay.

The calcium balances of the four groups were as follows: IV > II > I > III; whereas, according to the percentages of calcium utilized, the groups ranked I > IV > II > III. A marked depression in calcium retention was found in the animals receiving the $CaCO_3$ + $MnSO_4$ supplement as compared to the other groups, which may be attributed to the manganese sulfate. Group IV, receiving the additional trace elements, showed a distinct advantage in the maintenance of a positive calcium balance over the groups receiving the other supplements.

The earlier work showing that cows utilize calcium more efficiently when fed a low calcium ration than when the ration is high in calcium and that the retention is also governed by the animal's demand for calcium was substantiated in this study.

No appreciable differences were found in the hemoglobin content, red blood cell counts, red blood cell volumes, the concentrations of plasma proteins, calcium and inorganic phosphorus of the 4 groups.

FEEDING AND MANAGEMENT

P30. Needed Dairy Feed Ingredient Research. RAYMOND T. PARKHURST, Flory Milling Co., Inc., Bangor, Pennsylvania.

During the periods of shortages in recent years, most feed manufacturers and mixers have not had sufficient information concerning several new ingredients offered to know at what levels they could be used, if at all.

This paper will offer for discussion data concerning several ingredients about which more information is needed and which offer possibilities as either emergency or regular constituents of dairy feeds.

P31. Ground Ear Corn as Compared to Ground Shelled Corn in a Simple Grain Mixture for Milk Production. C. F. MONROE AND W. E. KRAUSS, Ohio Agricultural Experiment Station, Wooster, Ohio.

A simple grain mixture containing ground ear corn was compared to one containing ground shelled corn. In addition to the corn both contained soybean oil meal, with 2.5 per cent more being added to the ground ear corn mixture in order to equalize the protein content of the two mixtures.

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* Mico, manufactured by the Limestone Products Corporation of America, Newton, New Jersey, consists of calcite flour fortified with Mn, I, Co and Cu.

Comparable amounts of salt, steamed bone meal, and limestone were included in the two mixtures.

Corn silage was fed at rate of 30 pounds daily per cow. Legume-mixed hay was fed in liberal amounts, but it was not weighed.

Two reversal feeding trials of 100 days each were conducted. Seventy Holstein cows, fed and milked three times daily, were used in the work. Of this number 16 participated in both trials. The data for these cows are considered as a third trial.

The three trials are in general agreement. Milk and butterfat production were a little higher on the ground shelled corn mixture. In terms of 4 per cent milk the production on the latter amounted to 98.83, 98,29 and 98.62 per cent of the former in the three trials, respectively. An analysis of the productions following the reversing of the feeding at the end of the first period in each trial confirms the above findings.

There were no marked differences in liveweight gains on the two mixtures.

The butterfat tests were apparently not affected by the form in which corn was fed.

A replacement value for ground ear corn (or cobs) in terms of ground corn cannot be determined because of the additional amount of soybean meal in the ground ear corn mixture and because the amounts of hay consumed are not known.

The financial returns were a little higher on the ground ear corn mixture than on the ground shelled corn mixture. The difference as calculated according to prices prevailing in 1946, amounted to 48 cents per month per cow.

It is concluded on the basis of this work that the common practice of feeding ground ear corn rather than ground shelled corn is justified.

These trials furnish additional evidence on the possibility of using the simple grain mixture. The 16 cows that were in the two trials from November 1 to May 8, a period of 190 days, averaged 42.9 pounds of milk daily. On a 30-day basis they averaged 1287 pounds of milk and 42.23 pounds of butterfat. Likewise, the production of the other 54 cows used in this work represents 100 days of continuous feeding. The average production of these cows was almost 47 pounds of milk daily.

P32. **Ten Years Evaluation of Bluegrass Pasture.*** H. B. MORRISON AND FORDYCE ELY, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

Dairy heifers (Holstein and Jersey) were used to graze an 11.6-acre bluegrass pastures at Lexington, Kentucky, during the 1936 to 1945 seasons, inclusive. No other feed was provided them while on pasture, except salt and bonemeal. The heifers were weighed on three consecutive days at two-week intervals. The pastures were grazed as closely as possible without overgrazing. Heifers were added or removed when necessary following each bi-weekly weighing period, in accordance with the conditions of the pasture.

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* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by the permission of the Director.

Pasture yields are expressed in pounds of T.D.N. per acre. An allowance of 3.53 pounds of T.D.N. was made per pound of gain and 2.73 pounds deducted per pound loss in weight of the heifers. Credit for maintenance was calculated at the rate of 7 pounds of T.D.N. per 1000 pounds weight per day.

The length of the pasture season varied from 168 to 224 days and averaged 195 days.

The average yields of T.D.N. per acre by months (extremes in parentheses) were as follows: April 195 (0–626), May 535 (346–717), June 464 (278–633), July 295 (82–484), August 291 (117–432), September 263 (104–481), October 162 (0–378) and November 20 (0–72).

The highest yield per acre of T.D.N. in a season was 2786 pounds (in 1938), the lowest 1343 pounds (in 1936), and the 10-year average yield was 2226 pounds T.D.N. per acre.

The average yield per acre of this pasture (2226 pounds T.D.N.) is the equivalent of the T.D.N. furnished by 49.5 bushels of corn or 2.2 tons of alfalfa hay. The labor involved in production of this pasture was far less than that involved in the production of corn or alfalfa hay, while the T.D.N. yield of the pasture is fairly comparable with that of corn and alfalfa hay in this vicinity.

P33. Silage from Drought-Damaged Corn. C. F. MONROE, A. E. PERKINS, C. E. KNOOP, AND LOUISE SKINNER, Ohio Agricultural Experiment Station, Wooster, Ohio.

Corn silage made from drought-damaged corn was found to have approximately the same chemical composition and feeding value as a silage made from "normal" corn. Apparently the unfavorable growing conditions had resulted chiefly in a reduction in total yield and in the quantity and quality of the ears produced. Although the so-called "normal" corn was not up to the usual standard, it had been less affected than the other corn, probably as a result of a difference in the soil conditions.

A large percentage of the ears on the drought-damaged corn was denuded of husks at the tips. On many of these ears a fungus identified as ***Fusarium moniliforme*** was present, together with an infestation of black beetles and small larvae of the species ***Carpophilus lugubris*** Murr. There was some question regarding the effect of these conditions on the quality of the silage.

In a feeding trial with milking cows the two silages appeared to be equally palatable. The experimental feeding plan called for a near minimum use of hay and grain with a maximum use of silage. Under these conditions no significant differences in the production from these two silages was shown.

P34. The Unknown Lactation Factors in Corn Silage. C. F. HUFFMAN, S. T. DEXTER, AND C. W. DUNCAN, Michigan Agricultural Experiment Station, East Lansing.

Lactating cows fed alfalfa hay alone become depleted in unknown lactation factors. The replacement of a part of the T.D.N. with an equal amount of T.D.N. in grain results in an increase in the production of 4 per cent fat-corrected milk. Grains such as corn and wheat furnish factors which are lacking in the hay. Corn silage made from varieties of corn grown for grain contains considerably more grain than ordinary corn silage. Silage made from this variety of corn is in reality a mixture of corn stalks and corn grain.

In the first experiment corn silage made from an ear corn variety ensiled at the early dent stage was used as a partial replacement of hay in the ration of the depleted cows. When the replacement was made with hay and corn silage so that the T.D.N. was equal to or less than with hay alone there was a 19 per cent increase in 4 per cent fat-corrected milk. In 10 comparisons there was a 24 per cent increase in fat-corrected milk per pound of T.D.N. when a part of the hay was replaced by corn silage. There was also a 41 per cent increase in fat-corrected milk produced per pound of dry matter.

The data indicate that the grain in corn silage should be considered in calculating the amount of grain to be fed to milking cows. The use of the same "thumb" rules for feeding with or without corn silage may result in the overfeeding of grain when corn silage is fed.

In a second experiment, silage from corn with very few ears was compared with silage from an ear-producing variety of corn for milk production. The ear-corn silage was superior to the silage-corn silage for milk production on the dry basis.

P35. A Preliminary Report on the Study of Factors Influencing Rumen Microflora. A. L. BORTREE, K. M. DUNN, R. E. ELY, AND C. F. HUFFMAN, Michigan State College.

Microscopic counts were made of the microflora of the rumen contents of dairy animals to determine the effect of various feeds on the numbers of microorganisms present.

Rumen samples were taken prior to feeding and at two-hour intervals after feeding for periods of ten to twelve hours. The samples were preserved in formalin, stained with Gram's iodine, and counted in a Petroff-Hausser counting chamber. Only the larger microorganisms which stained deep blue with iodine were counted; therefore the results reported do not necessarily reflect the changes in the total population.

The results which have been obtained to date indicate that there is a rapid increase in the numbers of these organisms present in the rumen within two hours after the animals have been fed and that these high counts are maintained or increased for several hours and then gradually return to the range observed prior to feeding.

The quality of the roughage fed appeared to influence the bacteria counts somewhat but the changes were not marked. Likewise when the animals were changed from hay to pasture the changes in the counts were not great.

When three pounds of glucose was given in addition to the usual feeding of hay, the counts were about 100 per cent greater than those observed for hay alone. The administration of starch under similar conditions produced results which were only slightly different from those obtained with hay alone.

P36. The Physiological Role of the Rumen of the Young Bovine as Indicated by the Growth and Blood Composition of a Rumenectomized Calf.

G. H. WISE, R. P. LINK, W. W. THOMPSON, AND M. J. CALDWELL, Kansas Agricultural Experiment Station, Manhattan.

Rumenectomy was employed as an indirect means of determining the nutritional role of the rumen in the young calf. This organ was removed at the rumeno-reticular groove, using a technique similar to that developed by the Pathology Department of the Ralston Purina Company (*private communication*). The operation was preformed on four calves, two of which succumbed to post-operative shock. The remaining two were maintained under standard dietary and managerial regimes until the health of the calves indicated the need of corrective measures.

One of the calves developed normally, manifesting no ill effects except a marked tendency to bloat, particularly when grazing spring grasses. The chronic nature of the case suggested an impairment of the rumen activity. Autopsy of the sacrificed calf revealed marked regeneration of the rumen, which was normal in total size but malformed and deficient in the posterior region including the dorsal and ventral sacs.

The second calf manifested no abnormalities during the two months immediately following the operation but subsequently became increasingly unthrifty, revealing a waning appetite and a marked retardation of growth. Hematological examination of the blood disclosed marked anisocytosis and poikilocytosis, and analysis of the serum indicated avitaminosis A. Vitamin A therapy increased the level of this constituent in the blood but failed to alter any of the other abnormalities. Subsequent daily administrations of ruminal fluids stimulated the appetite, accelerated growth, increased the vigor, improved the general appearance and reduced the number of poikilocytes of the blood.

After 74 days, the foregoing therapy was replaced with spring pasture. The weight gains were greatly reduced but otherwise the calf continued to improve. The reduction of gains probably was the consequence of inadequate energy intake, since the feed capacity of the stomach appeared to be subnormal.

The increased appetite seemed to stimulate redevelopment of the rumen as evidenced by increased distention and by the initiation of rumination. The appearance of considerable undigested material in the feces, when the initial increase of the appetite was noted, indicated abnormal digestion. This was gradually ameliorated, presumably as a result of rumen development.

These observations further emphasize the recognized role of the rumen in the formation of products essential for the maintenance of the health of the calf.

P37. Effect of High Humidity and High Temperature on Dairy Cows.

D. M. SEATH AND G. D. MILLER, Louisiana Agricultural Experiment Station.

Observations made of Holstein and Jersey cows during the summers of 1944 and 1945 showed that changes in air temperature were the major cause for changes in both body temperature and respiration rate of milking cows. A statistical analysis of the data indicated that a change of one degree in air temperature had from 13 to 15 times as much influence on body temperature as did one per cent change in relative humidity. Likewise, one degree change in air temperature affected respiration rate 41 to 43 times as much as did a one per cent change in humidity.

Partial correlations between air temperature and body temperature (humidity held constant) were 0.674 and 0.534 for the two years; whereas, relative humidity with body temperature partial correlations (air temperature constant) were 0.16 and 0.10. On this same basis air temperature and respiration independent of humidity had partial correlation of 0.748 and 0.353, while humidity and respiration rate (with air temperature constant) had only very small negative correlations, – 0.06 and – 0.02.

Air temperature and pulse rate independent of humidity had only small partial *r* values, 0.195 and 0.159. Humidity and pulse rate independent of air temperature had insignificantly small *r* values, *i.e.,* – 0.05 and 0.07.

P38. The Causes and Extent of Variations in Weight of Dairy Cows Weighed at Half-hour Intervals over Ten-day Periods. N. N.

ALLEN, Vermont Agricultural Experiment Station (Now at University of Wisconsin).

In order to study the causes and extent of fluctuations in live weight, dairy cows and heifers were weighed at half-hour intervals over periods of ten days, with detailed accounting for their intake of food and water, and outgo of feces, urine, and milk. This was accomplished without disturbing the usual routine of the animals by confining them to stalls mounted on the platforms of scales. Five milking cows, 3 dry cows and 2 heifers were included.

The milking group averaged 990 pounds live weight; 62.8 pounds of feces and 16.3 pounds of urine eliminated; and 28.2 pounds of milk produced daily.

The non-milking group averaged 1043 pounds live weight; and 40.4 pounds of feces and 12.1 pounds of urine eliminated.

The milking group consumed an average of 51.0 pounds of feed and 85.3 pounds of water daily, as compared to 39.2 pounds of feed and 40.0 pounds of water for the non-milking group. The unaccounted-for loss averaged 29.4 pounds for the milking group and 26.6 pounds for the non-milking group.

While the heavier milkers showed a much wider range of variation in weight within the day due to greater feed and water consumption, and correspondingly greater weight losses, the trends of the changes were very similar. The lowest weights were recorded quite uniformly at the 5 o'clock weighings immediately prior to feeding, with little choice between the morning and evening low values. The range of the day-to-day variation was narrowest at the time when the weight was lowest. The maximum average weight within the day occurred 2½ hours after the evening feeding. While the same amount of feed was consumed in the morning, more time was taken to eat it, and the offsetting losses were greater, consequently the weight did not reach as high a peak.

There were four times each day when a single weighing accurately represented the average for the day. Two of these occurred shortly after the two feedings when the weight was increasing rapidly. The other two occurred during the periods of declining weight when the rate of change was slow. Because of the slow rate of change, the weighing time should be selected during the declining phase to secure a weight with average fill. Under the conditions of this experiment, 1:00 A.M. and 11:30 A.M. were the points at which the average was reached.

These data suggest that careful thought should be given to the time and manner of weighing, keeping in mind the purpose for which the weights are to be used.

HAY

P39. Standardizing Hay Palatability Trials with Calves. H. S. WILLARD, Agricultural Experiment Station, University of Wyoming, Laramie, Wyoming.

A study of the hay consumption of 34 Holstein calves from 3 to 12 months of age showed that consumption followed hay allowance over a wide range of weight and age. When hay allowance was increased by one pound daily the coefficient of variation in consumption of three types of hay ranged from 1 to 3 per cent, the standard deviation ranged from 0.4 to 0.8 and the standard error between 0.05 to 0.2.

This study indicated significant variations in consumption of individual calves. Additional variations of individual calves were revealed in a study of the consumption of 1st and 2nd cutting alfalfa fed over a 10-day period to six calves each of different weight. A high, but not significant, correlation was shown between live weight and hay consumption.

Rules are formulated for standardizing palatability tests which should give comparable results year after year.

P40. Comparative Value of Alfalfa, Mixed Grass and Legume, and Timothy Hays for Young Dairy Cows. C. L. NORTON, Department of Animal Husbandry, Cornell University, Ithaca, New York.

Continuous feeding trials were conducted for the purpose of determining the comparative feeding value of alfalfa, mixed grass and legume, and timothy hays. Three experimental groups of 8 Holstein heifer calves each were used. The experimental period was from birth to 20 weeks of age.

The calves were raised according to the Cornell limited whole milk and dry calf starter system. A total of 350 pounds of whole milk was fed to weaning which was at 7 weeks of age. Calf starter was fed free choice up to a maximum consumption of 4 pounds per head daily. Hay was fed ad libitum and weighbacks were taken daily.

As the calves were born they were assigned in rotation to the various treatments. A total of 24 calves was used in the experiment.

A summary of the growth and feed consumption was made at the end of 20 weeks. Expressed in terms of per cent of Ragsdale's normal, the gain from birth to 20 weeks of age for the calves fed the three hays was as follows: Alfalfa, 108.1 per cent; mixed grass and legume, 102.4 per cent; and timothy, 95.7 per cent. The apparent differences in rate of gain were not statistically significant.

Although the differences in the consumption of calf starter from birth to 20 weeks of age were not significant statistically, a definite trend was suggested. It was observed that the calves receiving timothy hay and mixed grass and legume hay ate starter at the maximum rate more quickly than the calves receiving the alfalfa hay. Greater differences might have been obtained if the calf starter intake had not been limited to 4 pounds per head daily. The groups ranked in the following order on the basis of total calf starter consumed: Timothy, 339.5 pounds; mixed grass and legume, 391.8 pounds; and alfalfa, 368.8 pounds.

There were large, rather consistent differences among the three groups in the total consumption of hay up to 20 weeks of age. The groups ate the following amounts of hay during the experimental period: Alfalfa, 492.4 pounds; mixed grass and legume, 421.3 pounds; and timothy, 326.0 pounds. These differences were statistically significant.

The data indicate that there was an inverse relationship between the amount of hay consumed and the amount of calf starter eaten. The calves receiving alfalfa hay ate the most hay and the least calf starter of the three groups. The calves receiving timothy hay ate the least hay and the most calf starter.

P41. Feeding Barn Dried Hay to Dairy Heifers. C. E. WYLIE, JOHN EWING, AND S. A. HINTON, University of Tennessee.

Great advances have been made in the use of the barn hay drier as a means of improving the quality of hay, and in saving more hay. Little work has been done in testing the feeding value of this hay with dairy animals. In order to check on the growth of Jersey heifers fed barn dried hay, feeding trials were set up by the Tennessee Experiment Station at Knoxville and Columbia. The rations were as follows: Group I—Long field cured alfalfa hay ad lib. Group II—Long barn dried alfalfa hay ad lib. Group III—Chopped barn dried soybean hay ad lib.

The heifers were fed hay in groups with records kept of the weights of hay put into the manger and hay rejected. The original hay was graded and chemically analyzed. Rejected hay was chemically analyzed.

The growth of the heifers was checked by weights and heart girth measurements, and compared with normal growth.

The heifers seemed to make normal growth and to be thrifty in appearance. The details of feed consumption and growth are shown in table form. As the amount of hay was increased in the manger, the rejected hay increased in amount. When the feeding level was so low that little feed was rejected, the intake was below the (Morrison) feeding standard.

P42. Comparative Efficiency of Three Methods of Harvesting and Preserving Forage Crops. R. E. HODGSON, J. B. SHEPHERD, L. G. SCHOENLEBER, H. M. TYSDAL, AND W. H. HOSTERMAN, Division of Nutrition and Physiology, Bureau of Dairy Industry, Divisions of Engineering and Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Re-

search Administration; and the Grain Products Branch, Production and Marketing Administration.

Alfalfa was made into wilted alfalfa silage, barn-cured hay and field-cured hay to compare the relative efficiency of the three methods of harvesting. The alfalfa was cut when one-tenth to one-fourth in bloom and harvested in the three ways simultaneously. Both the first and second crops were so harvested. Except for about one-half of the first cutting field-cured hay, the alfalfa was put up without rain. Records were kept of the labor and machinery required to harvest the crops and the losses in dry matter and nutrients and the changes in the composition of the forages were determined. The three forages were fed to milking cows to compare their values for milk production and the effect on the vitamin A value of the milk.

No appreciable differences were observed in total man hours or machinery hours required for harvesting when compared on the basis of a ton of dry matter saved for feeding. The wilted silage method was the most efficient of the three in conserving dry matter, protein and carotene for feeding. The barn curing method was the next most efficient. Of the original yields of dry matter, 80 per cent and 86 per cent were available for feeding when the first and second cutting were made into silage; 80 per cent and 79 per cent respectively when they were made into barn-curd hay; and 69 and 77 per cent respectively when they were made into field-cured hay. Of the original yields of protein in the second cutting, 84 per cent was saved for feeding with the silage method; 79 per cent with the barn-curing method; and 70 per cent with the field-curing method. The silage contained 12.7 per cent more protein and the barn-cured hay contained 9.6 per cent more protein than the field-cured hay.

The carotene in the dry matter of the first cutting used for silage averaged 278 micrograms per gram when cut, 239 when ensiled and 156 when fed; that barn-cured averaged 286, 143 and 50 respectively when cut, when put on the drier and when fed; and that field-cured averaged 270, 58 and 9 respectively when cut, when stored and when fed. The carotene in the dry matter of the second cutting used for silage averaged 308 micrograms per gram when cut, 218 when siloed and 82 when fed; that barn-cured averaged 303, 122 and 22 when cut, when put on the drier and when fed; that field-cured averaged 302, 50 and 12 when cut, when stored and when fed.

The first cutting wilted alfalfa silage fed with corn silage and concentrates caused cows to produce butterfat containing over 18,000 I.U. of vitamin A per pound continuously throughout a 110-day winter feeding period. Other similar cows fed U. S. No. 2 leafy alfalfa with corn silage and concentrates produced butterfat that after 20 days averaged 16,533 I.U.; after 50 days, 12,450 I.U.; after 80 days 9,927 I.U.; and after 110 days 9,190 I.U. per pound.

P43. Nutritive Value of Barn-Cured Hay. K. L. TURK AND C. L. NORTON, Department of Animal Husbandry, Cornell University, Ithaca, New York.

Feeding trials have been conducted to compare the feeding value of barn-cured hay with hay cured in the field baled with a windrow baler, and with field-cured hay stored in a long condition. Results of two years' experiments are reported.

In an 18-week reversal study, 15 Holstein cows were fed each of the three hays for a period of six weeks. There was no transition between experimental periods. The hay, a mixture of grasses and legumes, was the only roughage and was fed in amounts that allowed some weighbacks. Grain was fed at an arbitrary declining rate.

A summary of the milk production showed only slight differences between the three hays. When fed the experimental hays, the cows produced the following daily amounts of 4 per cent F.C.M.: barn-cured, 30.0 lbs.; field-cured baled, 29.3 lbs.; and field-cured long, 28.9 lbs. However, the palatability of the hays as judged by the pounds consumed daily assumed a different order: field-cured long, 33.5 lbs.; barn-dried, 32.7 lbs.; and field-cured baled, 30.8 lbs. These differences were not of sufficient magnitude to assume any practical importance. The barn-cured hay, however, had a noticeably greener color, a slightly higher carotene content, and a consistently higher official grade.

A second year's trial using first cutting and second cutting alfalfa hay gave similar results. There was no consistent advantage for any one treatment in the amount of hay consumed per head per day, nor in the amount of 4 per cent F.C.M. produced daily. Carotene values showed an advantage for the barn-cured hay over the field-cured hays. Federal grades were uniformly higher for the barn-cured hay.

P44. A Still Unidentified Nutrient in Roughages and in Milk. A. M. HARTMAN AND C. A. CARY, Division of Nutrition and Physiology, Bureau of Dairy Industry, Agricultural Research Administration, United States Department of Agriculture.

A still unidentified nutrient, X, occurs in milk, skim milk, commercial skim milk powders, cheeses, and certain other foods and feeds. Casein preparations may be excellent sources of X or be entirely deficient in it, depending upon how they are made. When rats depleted of X are placed on diets deficient in X but adequate in respect to all known nutrients, their rates of growth, development and fattening are decidedly retarded. With diets of this sort containing 25 per cent or 45 per cent of protein such rats grow at rates that are, respectively, only 54 per cent and 25 per cent of that of their sex-litter mates receiving X as a supplement to such a diet; and rats receiving 65 per cent of protein generally fail to survive on such a diet deficient in X, whereas their sex-litter mates receiving X survive and make good growth.

X is not present in whole wheat flour, white flour, enriched white flour, wheat bran, corn meal, soybean oil meal, linseed oil meal, heat-coagulated egg-white or yeast. Besides occurring in milk and the milk products referred to above, X occurs in bluegrass, alfalfa and alfalfa hays, timothy hays, lettuce, egg yolk, beef muscle and pork muscle. The X potency of milks produced by cows on different barn rations and on pasture have been compared.

P45. Fan-Ventilated Long Hay; A Chemical Study. A. E. Perkins, Louise Skinner, and R. G. Washburn, Ohio Agricultural Experiment Station.

During both the 1944–45 and 1945–46 seasons, second cutting mixed alfalfa was fan-ventilated in a small mow at the dairy barn of the Ohio Agricultural Experiment Station in cooperation with the Department of Agricultural Engineering. A home-built fan which proved relatively inefficient was used the first season. This was replaced by a more efficient fan and an improved form of duct in the later test.

In addition to dry-matter determinations to ascertain the progress of drying, chemical analyses including determinations of total sugars and carotene as well as the usual feed analysis were made at each stage of the operation from standing crop to hay as fed.

The hay in both seasons was fed to dairy heifers in a palatability comparison, with field-cured hay from the same crop 1944–45, and with the best commercial baled hay locally obtainable 1945–46.

Three larger successive batches of hay were also fan-dried in another barn over a single installation, by baling out the first two lots. No feeding comparison was made with this hay though all the lots were judged to be of high quality. The workmen and feeders, not always too friendly to innovations, were favorably impressed with the hay. Chemical studies were likewise made covering all stages of the operation.

In the first season's operation, the hay was stored at an average dry matter content of 52 per cent and the fan, in addition to being somewhat inefficient, was operated intermittently after the first few days. The resulting hay had a lower carotene and sugar content and was less palatable to the heifers than the field-cured hay from the same crop, made under highly favorable conditions, with which it was compared; but it was eaten when fed alone and was probably superior to most weather-damaged hay.

In the second season, both installations operated efficiently. Most of the lots were somewhat higher in dry matter when stored and the fans were run continuously until the hay a few inches beneath the surface had attained a dry matter content of about 80 per cent. All the hay dried under these conditions had contents of carotene and sugar comparing favorably with the field-cured portions of the same crop. One lot in particular which was fed in a palatability comparison was far superior to any hay with which it was compared. Its sugar content was well preserved and the carotene content, particularly toward the bottom of the mow, was remarkedly high—35–45 parts per million. Only the chemical results are presented in detail.

P46. The Comparative Feeding Value of Wilted Alfalfa Silage and Alfalfa Hay for Dairy Cows.

J. B. SHEPHERD, R. E. HODGSON, AND W. J. SWEETMAN, Division of Nutrition and Physiology, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Wilted alfalfa silage, barn-cured alfalfa hay and field-cured alfalfa hay harvested from the same fields at the same time during favorable weather were each fed to milking cows in a double reversal, double latin square design experiment using six groups of 3 cows each over three periods of 40 days each (10 preliminary and 30 experimental).

The wilted alfalfa silage as fed contained 40 per cent dry matter having 22.6 per cent protein and 82 micrograms carotene per gram. The barn-cured alfalfa hay was graded U. S. No. 2 leafy alfalfa clover mixed and as fed the dry matter contained 20.37 per cent protein and 22 micrograms carotene per gram. The field-cured hay was graded U. S. No. 2 alfalfa clover mixed and as fed the dry matter contained 19.26 per cent protein and 12 micrograms carotene per gram. On a dry matter basis, 11.2 per cent more of wilted alfalfa silage and 6.7 per cent more of barn-cured alfalfa hay were available for feeding than of field-cured alfalfa hay. The top third of the barn-cured hay was somewhat dusty in the central part of the mow.

The cows were fed all of each of these alfalfa roughages they would eat along with one and one half pounds of corn silage per 100 pounds liveweight daily and a 15 per cent protein grain mixture apportioned at the rate of one pound for each 3.5 pounds of 4 per cent fat-corrected milk produced.

As judged by alfalfa dry matter consumed in the preliminary periods, the wilted alfalfa silage was slightly more palatable than the barn-cured and field-cured hays which were about equally palatable. When fed wilted alfalfa silage the cows produced 3.1 per cent more 4 per cent fat-corrected milk, declined in milk production at a slower rate and made better liveweight gains, with slightly less grain, total dry matter and total feed nutrients than when fed barn-cured hay or field-cured hay. These results are in agreement with a similar experiment conducted last year in which cows produced 7.0 per cent more milk on wilted alfalfa silage than on field-cured alfalfa hay cut from the same field 3 days later and cured without rain, with 50-day declines in milk production of 6.7 and 20.9 per cent, respectively, when fed these feeds.

In the current experiment the cows produced about the same amount of milk but declined in milk production at a little slower rate and made better liveweight gains when fed field-cured alfalfa hay than when fed barn-cured alfalfa hay, consuming about the same amount of total dry matter and total feed nutrients.

On wilted alfalfa silage, barn-cured alfalfa hay and field-cured alfalfa hay, respectively, daily milk production (4% F.C.M.) averaged 32.8, 31.9, and 31.8 pounds; 30-day decline in production 4.3, 10.0, and 5.9 per cent; daily liveweight gains 0.47, 0.03, and 0.40 pounds; daily alfalfa dry-matter consumption 17.2, 17.2, and 17.5 pounds; daily corn silage dry matter consumption 4.9, 4.8, and 4.9 pounds; daily grain dry matter consumption 8.4, 8.8, and 8.6 pounds; and total dry matter 30.5, 30.8, and 31.0 pounds per cow daily. On all three rations the cows received considerably more protein and slightly more total nutrients than called for by the Morrison feeding standard.

The consumption of alfalfa dry matter and total dry matter per 100 pounds of 4 per cent fat-corrected milk averaged 52.4 and 92.9 pounds respectively on the wilted alfalfa silage ration; 53.9 and 96.5 pounds respectively on the barn-cured alfalfa hay ration and 55.0 and 97.4 pounds respectively on the field-cured alfalfa hay ration. Fed as above, milk production per acre of alfalfa forage (plus corn silage and grain) amounted to 16 per cent more on the wilted alfalfa silage ration and 9 per cent more on the barn-cured alfalfa hay ration than on the field-cured alfalfa hay ration.

BREEDING

P48. Crossing Zebu with Northern Breeds of Cattle in Jamaica.

JOHN W. HOWE, Iowa State College, Ames, Iowa.

Purebred dairy cattle of the breeds raised in the United States, when raised under tropical conditions show lack of growth and development, combined with a lowered milk production. High mortality is caused from tropical diseases, to which such animals are susceptible. To develop an animal resistant to such conditions, the Jamaica Department of Agriculture in 1921 started crossing the Sahiwal breed (imported from India) with the Jersey, Guernsey and Holstein breeds. Jersey crosses, having from ¼ to ⅛ Zebu blood gave the most satisfactory results in both production and resistance to environment.

P49. Some Experiences with Central Artificial Breeding Organizations.

H. A. HERMAN, W. K. DINWIDDIE, AND M. J. REGAN, University of Missouri.

Five years experience with a *central* or *federated* dairy cattle artificial breeding organization in Missouri has indicated that this type of project is well adapted to large-scale operations and has many advantages from the standpoint of economy, flexibility, and efficiency over smaller local breeding associations. At the present time 3 such organizations, involving 55 local units, and servicing some 50,000 cows annually are in operation. A fourth organization is in the process of formation. One of these organizations is a cooperative, one a semi-cooperative, and the third is privately fostered. This last-named organization, The Mid-West Breeding Farms, Trenton, Missouri, is owned and sponsored by the Central Farm Products Company, Trenton, Missouri, in coöperation with the Missouri College of Agriculture. This organization is in its fifth year and was set up to provide approved sire service in an area where the trend is definitely toward dairy and livestock farming. All inseminators are well-trained laymen who work on a salary and a bonus system. A veterinarian employed by the company to aid in all herd health and technical problems is available. All sires, 12 to 15 in number, including several proved sires, are approved by the Missouri College of Agriculture. A summary of field results for 10 local units of this organization from February 1943 to June 1945 reveals that during this period 6052 cows were serviced. A total of 8968 services were required with an average conception rate of 1.8 services. About 86 per cent of the cows settled the first 3 services. Cows serviced the first time in December, January, and August required the largest number of services. The spring months were the most favorable. For 16 sires studied the conception rate ranges from 1.6 to 2.4 services per calf. In local units the average conception rate varies from 1.5 to 1.9 services. Semen collections were made at an average of 5 times per month. Some sires were occasionally used 7 to 8 times per month. The initial motility of the semen averaged 3.7 and was diluted at the ratio of 1: 8. The average storage time was 34 hours. The number of cows settled per year per sire ranged from 150 to 414 with an average of approximately 300 for the average sire in full service. If all of the semen had been utilized, several times this number could have been settled per sire. With the expansion from 10 to 19 units a more efficient use of semen will naturally result. This organization was not founded with the idea of making profits. Financially the project has been able to pay rent on the barns and land where the bulls are housed, feed and operating expenses, salaries, bull replacement costs, etc., but has otherwise returned no startling dividends. The service fee is $6.00 per cow. Patrons of the Central Farm Products Company receive a refund of $2.00 as a means of encouraging greater participation in the use of better sires.

P50. The Use of Pregnancy Diagnosis with Artificial Breeding.

L. E. CASIDA, G. R. BARRETT, AND C. A. LLOYD, University of Wisconsin.

Pregnancy diagnoses were made on the cows inseminated in the Wisconsin Experimental Breeding Project during the months of February to May and September to December, 1945, inclusive. The method of Wisnicky and Casida (unpublished), which depends on palpation of the amniotic vesicle, was used beginning at 34 days after breeding and the majority of the diagnoses were made before 50 days after breeding. For 3,099 inseminations, a comparison of the fertility as determined by palpation with the fertility as estimated from the herd books, based on "non-return" information, showed a discrepancy of 15.3 per cent at 30 to 60 days, 6.1 per cent at 60 to 90 days, and 3.0 per cent at 90 to 120 days. These data do not include 78 additional inseminations on which no information was obtainable but which would have been considered 100 per cent pregnant on the basis of "non-return" information. An analysis will be presented of the homogeneity of these discrepancies in relation to month and season of the year and number of previous services on the individual cows. Evidence was obtained indicating that at least 4 per cent of the diagnosed pregnancies were lost before the end of 4 months.

P51. The Effect of Certain Coal-Tar Dyes Used for Semen Identification on the Livability and Fertility of Bull Spermatozoa. JOHN O. ALMQUIST, Pennsylvania Agricultural Experiment Station, State College, Pa.

In the routine use of artificial breeding of dairy cattle it is impossible to distinguish the semen of one breed from that of another by any characteristic exhibited by the semen itself. Studies were made to determine whether certain coal-tar dyes could be used to color diluted semen in order to reduce errors in its handling, particularly in those instances where semen of one breed might inadvertently be used to inseminate cows of a different breed.

Preliminary studies indicated that coal-tar dyes obtained commercially in liquid form offered a simple means of coloring semen under field conditions. The aqueous solutions of coal-tar dyes used in these studies were certified by the U. S. Food and Drug Administration and required no further preparation before use. These certified liquid dyes are standardized as to strength, uniform as to color intensity and can be stored for long periods of time. Since semen diluted with egg yolk-citrate is yellow in color, it was necessary to resort to the use of shades or tints. The following certified solutions of coal-tar dyes as prepared by the Warner-Jenkinson Manufacturing Company of St. Louis, Missouri, were found to give adequate color differentiation when added to diluted semen: (1) strawberry shade red color (pure dye content not less than 5.2%), (2) emerald shade green color (pure dye content not less than 2.7%), (3) purple shade grape color (pure dye content not less than 4.1%) and (4) brown color (pure dye content not less than 3.7%). Chemically these colors are blends of various azo, pyrazolone, and triphenylmethane dyes.

To determine the influence of these dyes on the livability of spermatozoa, semen samples diluted at the rate of one part of semen to 10 parts of egg yolk-citrate diluter were colored using one drop of dye solution for each 10 ml. of diluent. A total of 15 semen samples were stored at 40° F. and the percentages of motile spermatozoa were determined every 2 days for a period of 10 days. Analysis of variance involving 375 motility estimations showed a significant difference ($P = < 0.05$) between treatments. Highly significant differences ($P = < 0.01$) between ejaculates and between storage intervals, as well as a highly significant storage interval × ejaculate interaction were found. The red and green color preparations significantly reduced spermatozoon livability while the means for semen colored brown and purple were not significantly different from the mean of the untreated control.

After the foregoing experiments upon spermatozoon livability were completed, a study was conducted to determine the effects of these color preparations upon the fertility of semen used under routine artificial breeding conditions. A field trial was conducted utilizing 10 semen samples from each of 5 bulls in a Latin square experimental design involving the 4 previously described color reagents with proper controls. In this experiment a lower concentration of dye was employed; thus one drop of the red, green and brown shades was added for each 25 ml. of egg yolk-citrate diluter (or 0.2 ml. of dye solution per 100 ml. of diluter) while one drop of the purple shade was added for each 15 ml. of diluter. These concentrations provided adequate color distinctions. A total of 2,995 inseminations, of which 2,376 were first services and 619 were second services, were involved in this experiment. When data based on the percentages of first and second service cows which did not return for service within 60 days were used, no statistically significant differences were found in the fertility of the colored and uncolored semen samples.

P52. The Effect of Feeding Potassium Iodide and Skim Milk Powder on Spermatogenesis. C. E. KNOOP, Ohio Agricultural Experiment Station, Wooster, Ohio.

The effects on spermatogenesis with certain problem bulls in artificial breeding work were noted when a normal ration of legume or mixed hay and a regular concentrate mixture was supplemented by one of the following: A high protein feed containing kelp and fishmeal, iron and copper salts, iodinated-casein, shark liver oil, potassium iodide, skim milk powder, or a combination of potassium iodide and skim milk powder. Marked improvement in semen quality was observed in 7 of the 11 problem sires who daily received 50 mg. potassium iodide plus 0.8 pound skim milk powder for a period of 2 to 6 months. Improved spermatogenesis was indicated by one or all of the following factors: libido, quantity of semen, output of sperm cells, percentage of living sperm cells, and the motility-activity rating of the cells.

P53. Seasonal Effect on Spermatogenic Activity in the Bull and Reproduction in Cattle.

Ernest Mercier, Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

A yearly study of the semen characteristics of 10 bulls (5 Holstein and 5 Guernsey) used for artificial insemination in New York State revealed a highly significant breed difference only in respect to fertility.

Highly significant individual differences occurred for frequency of collection, volume, concentration, total sperm per ejaculate, methylene blue reduction time (M.B.R.T.), tailless heads, abnormals and fertility. The differences among bulls in the percentage of motile sperm was significant at the 5 per cent level of probability.

Highly significant monthly differences occurred for all the above-mentioned characteristics, except volume which varied at the 5 per cent level of probability and total number of spermatozoa which did not vary significantly.

A comparison between consecutive first and second ejaculates collected at a brief interval of time revealed a highly significant difference in respect to volume, percentage of motile sperm, concentration, total spermatozoa and tailless heads. The difference between ejaculates in the proportion of abnormal spermatozoa did not vary significantly.

A highly significant positive correlation occurred between the concentration and the fertility of 318 ungrouped ejaculations of these 10 bulls. In the same ejaculates, methylene blue reduction time and morphologically abnormal spermatozoa were negatively correlated with fertility at the 5 per cent level of probability.

The determination of the relationship between the characteristics of semen collected from 1942 through 1944 and the climatic factors for the corresponding period (temperature, barometric pressure, relative humidity, and length of day) showed that temperature and length of daylight are both negatively correlated with spermatogenic activity in the bull and reproduction in cattle under New York conditions. However, outside temperature and length of day are positively correlated with fertility of cattle under natural breeding in Eastern Canada.

P54. Time of Insemination and Conception Rate in Artificial Breeding.

G. R. Barrett and L. E. Casida, University of Wisconsin.

Owners of cows bred in the Wisconsin Experimental Breeding Project are asked to report the hour at which cows are first noticed in heat. This time and the time of breeding are recorded by the inseminator. Such information is available on 3,841 inseminations made during the periods from January 24 to May 31, 1945, and from August 13, 1945, to January 31, 1946. These have been grouped, according to the length of interval from notice of heat to time of insemination, into the following classes: less than 3 hours;

3 but < 6; 6 but < 9; 9 but < 16; 16 but < 20; 20 but < 25; 25 or more hours. The method of determining the fertility of inseminations is described by Casida, Barrett, and Lloyd in another paper at this meeting. The percentages of conception for the above groups are, respectively, 46.3, 55.8, 54.0, 53.5, 54.4, 50.5, 43.6 per cent, while the average for the entire group is 52.4.

P55. Artificial Insemination as a Means of Genetic Improvement.

J. L. Lush, Iowa State College.

Artificial breeding as compared with natural breeding may change the genetic composition of the next generation in either or both of two ways: (1) by decreasing the fraction of all males born which are to be used as sires, and (2) by improving the accuracy with which the breeding values of the potential sires are estimated at the time of deciding whether to use them or not. Artificial breeding, as compared with natural breeding, can make large changes in (1) but only small changes in (2). How much the rate of genetic improvement of the dairy population will be increased by these changes is appraised on the basis of what is known about: (a) the amount of genetic variability in the population, (b) the correlation between estimated and actual breeding values of sires, and (c) the amount by which the estimated breeding values of the selected sires exceeds the average breeding value of all males born.

P56. Deleterious Recessive Genes in Dairy Bulls Selected at Random.

S. W. Mead, P. W. Gregory, and W. M. Regan, University of California, Davis.

The background of the University of California breeding experiment with dairy cattle is presented, with a description of the original stock. Six unrelated sires, five Jersey and one Holstein, were selected at random so far as recessive genes were concerned and were tested for recessives by sire-daughter matings and other mating tests. Although some of the recessive genes carried by the sires may perhaps have escaped detection, there is no doubt concerning the eight that were positively identified.

Two lethal genes were found—one conditioning imperfect epithelium, the other an achondroplasia-like anomaly. The nonlethal genes were those conditioning proportionate dwarfism, flexed pasterns, strabismus, and congenital cataract. Two different forms of female sterility that prevented heifers from conceiving or producing offspring were also encountered. In the sterility found, only in animals of the Jersey herd, did all the affected heifers manifest normal heat periods. The sterility found only in the Holstein breed was associated with an abnormal estrous cycle, usually complete absence of heat. Both forms of sterility are sex-limited. There was no evidence that the fertility of heterozygous cows is impaired. One Jersey bull that was fully fertile was assumed to be homozygous for the Jersey type of female sterility. None of the sires proved were homozygous for the Holstein type of female sterility; but it is assumed that homozygous bulls are fertile. The expected proportions of sterility in heifers based upon the gene frequencies found are compared with the actual figures recorded in the literature.

The matings provided some data concerning the linkage relations of certain combinations of the mutant genes. The matings involving the combination of achondroplasia, strabismus, and flexed pasterns indicated that these genes are independent. Other matings indicated that cataract and the female sterility found in Jerseys are independent.

The feasibility of an organized program for amassing all kinds of genetic data on domestic animals is discussed.

THE FORTY-FIRST ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. Stoltz, *Secretary-Treasurer*

The American Dairy Science Association assembled in the Memorial Union at Iowa State College, Ames, Iowa, on Tuesday, June 18, 1946, at 9:30 A.M.

C. A. Iverson, Head of the Department of Dairy Industry, Iowa State College, introduced President Charles E. Friley, of Iowa State College, who gave the address of welcome.

Association President John A. Nelson was then presented and gave the following address:

THE PRESIDENT'S MESSAGE

It gives me great pleasure to address you at this forty-first annual meeting of the Association. The occasion affords additional gratification because it marks the twenty-fourth anniversary of my graduation from Iowa State College.

We are living in a changing world and the dairy industry is making its share of the changes. The direction these changes will take and how much they will benefit the dairy industry will depend very largely on the activity of each and every member of this Association. The American Dairy Science Association has a real responsibility to the industry it serves. More and more in the future the dairy industry must depend on the scientist to enhance the efficiency of dairy production, increase the efficiency of the processing of dairy products, discover new methods, new processes and new products that will tend to increase the consumption of dairy products. The scientist must aid in improving the sanitary standards and must aid in enhancing the quality of dairy products which have the kind of flavor that will appeal to more and more people. The future of the dairy industry will depend very largely on the amount and kind of research done and the application of this research in expanding the scope of the dairy industry field.

The biochemist, the bacteriologist, the geneticist, the nutritionist, the physiologist and research workers in other allied fields have made many valuable contributions to the dairy industry. In order to put the dairy industry in the strategic position which it rightly deserves, there is great need for continued emphasis on both practical and fundamental research in these fields. The future of the dairy industry will depend primarily on the kind and the amount of research done in the next few decades. The value of research can be more fully appreciated when it is considered that the industries making the most rapid progress are those most successful in solving research problems. Many commercial dairy concerns have established research work as a potential business development agency and by so doing have become leaders in the field.

It would be folly to list the research needed in the order of importance. However, there are some problems that have been aggravated by the war that need immediate attention. There are good indications that there will be a critical shortage of high-protein feeds for dairy cows in the near future. This means that the agricultural experiment stations must find substitutes for the more common dairy feeds if milk production is to be held up to a point where there will be sufficient dairy products necessary in the diet.

In view of the post-war shortage in dairy feeds, an agricultural experiment station in the Pacific Northwest conducted experiments with ground wild yellow mustard seed and ground fanweed seed as concentrate feeds for milking cows. It was found that these weed seeds compared favorably with soybean oil meal as a protein supplement to the grain ration. Contrary to the general belief, neither of these weed seeds had any detrimental effect on the flavor of the milk produced when properly fed after milking. Feeding trials have been conducted at the same station on substituting cull potatoes for part of the grain ration for milking cows. The results indicate that five pounds of potatoes can be fed in lieu of a pound of the grain concentrate. Organoleptic flavor evaluations of the milk produced indicated that potatoes do not impart an objectionable flavor to the milk produced. The findings of this station will be of great value to the dairy producers of the Pacific Northwest where these weed seeds are found in sufficient quantities as screenings from other grains and where large acreages of potatoes are grown. These experiments were mentioned only to indicate that this type of practical research can be done in other areas primarily to alleviate an approaching dairy feed shortage which no doubt will occur in all sections of the United States.

The discovery that the microorganisms in the rumen of the dairy cow can make protein from urea nitrogen and that urea can be fed to replace some of the high-protein feeds may have a real significance in the near future. Further investigations of this chemical product may prove it to be of a great and lasting benefit to milk producers. Further studies on the improved practices in harvesting roughages, especially alfalfa hay, would do much to help dairymen to produce more protein and other nutrients for the dairy ration. The foregoing are only a few of the practical research problems that are urgent and should be solved in the interest of the dairy farmer.

The time has come when more attention must be paid to milk production. The progress and growth of the dairy industry depends on efficient milk production. Efficient milk production depends upon the application of science to production problems. Researches on artificial insemination, the hormone thyroxine which has to do with the stimulation and intensity of milk and fat secretion, thyroprotein, calfhood vaccination, and fast or planned milking will all have a beneficial influence on increasing the efficiency of milk production if applied under the proper direction.

A discussion of the production phase of the industry would not be complete unless recognition is given to the accomplishments of the veterinarian and the animal pathologist and the part they have played in the progress made by the dairy industry. These scientists have tuberculosis well under control and are moving ahead with plans for the control of brucellosis. The next big step forward in animal disease control is to reduce the occurrence of mastitis. Some of the dairy production scientists have reason to believe that mastitis is primarily a herd management problem. The majority of the evidence seems to be on the side of those who believe it is a management problem. There is need for more research on mastitis. When more is known of the direct and indirect causes of this common udder affliction, definite progress can be made to lessen the occurrence of this disease which is greatly reducing the production in the majority of the dairy herds.

During World War II, considerable interest was placed on the vitamin A potency of butter. The preliminary report on the cooperative survey on vitamin A in butter issued by the USDA reveals that vitamin A potency of butters made in the different sections of the United States varies greatly by sections and also by seasons. The higher vitamin A content of summer butter over that made in the winter season is due no doubt to the higher beta carotene content in the summer feed. If the scientist could find ways and means to conserve more of the beta carotene in the feeds during harvest and storage, it would aid materially in increasing the vitamin A content of butter made in the winter season.

Dairy Herd Improvement Associations have been considered the most valuable project in dairy extension work. They have been of inestimable value in building up the production of dairy herds. Valuable as they are we have not been able to get more than a small percentage of the dairy cows in the United States tested under this plan. Dairy herd testing is the foundation of all good dairy practices. Dairy extension specialists should be encouraged to do all that can be done to get more cows under a testing plan. No milk producer can manage his herd intelligently unless he knows the production and the economy of production of every cow in the herd. This may sound too idealistic, but it would be a real step toward profitable dairying if the average fat production of the dairy cows in the United States would be twice what it is at the present time. That is a real goal for the dairy production extension specialist.

Teaching methods in the different dairy industry fields have undergone changes in the past two decades. The quality of teaching has been improved. Dairy course graduates have become leaders in their fields. Practical short courses have been given for the men working with the herds and in the plants. Short courses of the conventional type, from a few days to a few weeks duration, have done much to help put dairy science into practice. There is need for the enlargement of the teaching program. Many workers in the practical dairy field are not being reached. For example, since fast or planned milking has been introduced, practical instruction on how to carry out this milking procedure has not been given to many milkers. If fast or planned milking were practiced universally, the production of milk would be greatly increased.

It is gratifying to have the members of this association take an active part in the improvement of the quality of dairy products. The direction the dairy industry will take in the future will depend on what is done about quality. In the future, when dairy products become plentiful again, the market will change from a seller to a buyer market. Then the consumer will tell the industry the quality of product he demands and the plants with the high quality products will get increased sales. The plant that has quality has the confidence of the consumer. The plant that has the quality will have the kind of flavor in the product that the consumer desires.

The dairy scientist can do much for the industry in helping dairy plants with their sanitary problems. Plants are not only becoming more interested in sanitation, but they are actually showing aggression toward a cleaner, more sanitary and more attractive plant. Dairy manufacturers are beginning to realize that a step forward in sanitation is also a step forward in consumer confidence and confidence is needed to make any business successful.

Just previous to and during World War II, much was said about using more of the milk solids "below the cream line." The superior nutritive value of these milk solids has been fully recognized by nutritionists for some time. When non-fat milk solids are consumed in cheese, ice cream or other dairy products which contain them, they have consumer appeal. The main difficulty in getting more of these solids consumed as human food is to get them into a more appetizing form than that of non-fat dried milk solids to be used as such. From the standpoint of improved human nutrition and also from the standpoint of increased financial return to dairy farmers, the research worker can make a real contribution by continuing to develop new food and industrial uses for non-fat dry milk solids.

It is reported that about two-thirds of the annual production of slightly less than 300,000,000 pounds of non-fat dry milk solids produced before the war was used by the baking industry. It is known that non-fat milk solids have a number of advantages in baking, not only in the baking but in the increased food value and other desirable characteristics of the baked products. If the consumer knew how much the nutritive value of white bread is increased by the addition of 6 per cent non-fat dry milk solids, he would demand bread that contained these added solids. Considering the possibility of improving human nutrition and at the same time increasing the sale of milk solids, would it not be a good project for the dairy industry to cooperate with the baking industry to bring about a universal dry milk enrichment program for baked goods? Research that has been done and is being done seems to indicate that benefits other than increased nutritional value are also in the offing. This example is cited to point out what research in the right direction can do for the dairy industry, the baking industry, and for the consumers of bread products which includes a very high percentage of the population of the world.

There is a trend toward consumers' paying more attention to the flavor of foods. Many food manufacturers are making flavor appeals through magazine and motion picture advertising and radio broadcasts. The dairy industry has not fully realized the necessity of making flavor appeals to increase the sale of dairy products. There are examples in the dairy industry that go to show that flavor does have sales appeal. Milk dealers witness the sale of homogenized milk increasing at the expense of the sales of the same product that is not homogenized. Consumers forego the once popular cream line and the privilege of pouring off the top milk to be used as cream in order to get homogenized milk which has a more pleasing flavor than the same product would have if it were not homogenized. Observations like these bring out the desirability of doing more research on the improvement of flavor in dairy products. If nothing more is done than to teach milk producers the proper methods of feeding milking cows, there would be a general improvement in the flavor of the milk supply. The importance of feeding milking cows strong flavored feeds at the proper time so that their milk will be of good flavor has been demonstrated by some of the experiment stations. Experiments were conducted in which milking cows were fed eight per cent of ground fanweed seed in the grain ration immediately after milking without any detrimental effects on the flavor of the milk produced by the cows.

The American Dairy Science Association has a great future if we continue to work cooperatively and diligently. We have made progress during the past year. Secretary Stoltz will have a stimulating report to make to you at the business meeting on the progress of the Association the past year. We cannot endure long on the stimulation given us by a report. We must build to greater heights and to greater usefulness to the dairy industry. We should take interest in all activities large or small that will benefit the industry. We should act as advisors to dairy groups in our community. Our advice will do much to help improve the industry and put scientific methods and devices into practice in the community.

We have made a steady gain in membership but we must not become complacent on continuing to build up the membership of the Association. There is a cooperative creamery in the Pacific Northwest that has a slogan "Every Member a Field Man." That would be a good slogan for us if it were changed to "Every Member Get a New Member." Every thriving business must get new business to be successful. ADSA must continue to build up membership in order to be of greatest benefit to the industry it serves.

The year of tenure as President of ADSA has been an enjoyable one. Thanks to every member for your support and forebearance of all the mistakes or other sins of omission for no doubt there were many.

Mr. E. S. Estel, Editor of the Creamery Journal, Manager of the Dairy Cattle Congress, and Secretary of Iowa State Dairy Association, was then introduced and gave a very interesting address on the Dairy Industry. There were 296 members present.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Ames, Iowa, June 20, 1946

President Nelson called the meeting to order at 3:00 P.M. in the agricultural assembly room of Agricultural Hall. There were 163 present.

EDITOR'S REPORT

During the past year, twelve monthly issuses of the JOURNAL OF DAIRY SCIENCE were published. We regret that these issues did not always appear on schedule. However, their lateness in reaching your hands resulted from conditions beyond the editor's control.

Volume 28 of the JOURNAL OF DAIRY SCIENCE consisted of 996 pages of original articles, announcements, proceedings of the Annual Business Meeting and indices. This volume also contained 196 pages of Abstracts and abstract indices. The total number of Journal pages devoted to material other than advertising was 1,192.

Table 1 summarizes the Journal contents for volume 28; this table also includes similar summaries for volumes 26 and 27 for comparison.

TABLE 1

	Volume 26		Volume 27		Volume 28	
	No.	Pages	No.	Pages	No.	Pages
Manufacturing						
Original papers	59	542	40	356	51	484
Review papers	1	86	2	98		.. ...
Production						
Original papers	38	362	50	410	47	418
Review papers		...				
Miscellaneous		176		208		94
Total	98	1166	92	1072	98	996
Abstracts		264		208		196
Total pages printed		1430	...	1280		1192

Ninety-eight original papers were published; 51 of these dealt with research in the field of dairy manufacturing and 47 dealt with production subjects.

Again the editor gratefully acknowledges the generous assistance of a host of loyal workers whose help makes possible the publication of the JOURNAL OF DAIRY SCIENCE.

Upon motion duly seconded, the Secretary was requested to spread upon the minutes words of appreciation regarding the splendid work of our Editor and to adopt his report. The motion was carried unanimously.

SECRETARY-TREASURER'S REPORT

Membership and Circulation. The report of membership and the total circulation of the Journal for the past fiscal year, as well as for the first five months of 1946, is very encouraging. At the close of 1945, we had a total paid circulation of 2399 which is more than 100 higher than the total for 1944. Last year an error was made in the report of the total circulation because the new members were added into the total. This total should have read 2266 instead of 2377. As of June 1, 1946, our membership has exceeded any previous figure. We had 1534 members which is more than 100 more than we had at the end of last year and excels our previous high year of 1939. The following table gives you the total circulation as of December 31 for the past nine years and as of June 1 this year.

Dec. 31	Members	New members	Subscribers		Associate subscribers	Student affiliates	Total
			Domestic	Foreign			
1937	928		360	385	110	30	1813
1938	1101	332	352	395	125	81	2054
1939	1465	419	321	385	73	156	2400
1940	1339	105	331	360	62	283	2375
1941	1321	103	353	316	66	391	2447
1942	1276	100	371	212	49	235	2143
1943	1324	134	463	208	85	108	2188
1944	1388	111	519	233	81	45	2266
1945	1407	105	586	291	83	32	2399
June 1, '46	1534	215	579	310	85	162	2670

The following data is a summary of our loss and gain in members for the year 1945:

Membership, December 31, 1944		1388
Gain: New members, 1945	97	
Former student affiliates	8	
Total gain	105	
Loss: Members resigned and delinquent for 1945:		
Delinquent	35	
Resigned	32	
Deceased	1	
Servicemen	18	
Total loss	86	

Net gain in membership	19
Membership, December 31, 1945	1407

You will be interested to know that 72 per cent of our members pay their dues previous to January 1.

Following is a listing of our circulation by States as of June 1, 1946:

	Members	New members	Sub-scribers	Associate Sub-scribers	Student affiliates	Total
Alabama	5		3	1		9
Arizona	6	2	1			7
Arkansas	3	1	5	1		9
California	68	2	48	6	2	124
Colorado	10	1	5			15
Connecticut	17	1	7		1	25
Delaware	2		2		1	5
District of Col.	35	3	14	1	1	51
Florida	14	1	9			23
Georgia	20	9	10	1	1	32
Idaho	8		6			14
Illinois	149	17	47	2	5	203
Indiana	44	3	19	1		64
Iowa	33	3	8		67	108
Kansas	11		5	1		17
Kentucky	8		6	2		16
Louisiana	13	1	5			18
Maine	1		2	1	1	5
Maryland	40	2	9	1		50
Massachusetts	38	2	23	8		69
Michigan	54	5	13	8		75
Minnesota	50	6	12	4		66
Mississippi	10	2	2			12
Missouri	43	6	17	2	1	63
Montana	11		3		2	16
Nebraska	12	5	7		1	20
Nevada	1		1			2
New Hampshire	10	1	2			12
New Jersey	33	1	22	3		58
New Mexico	3		2			5
New York	142	17	82	8	2	234
North Carolina	14		8			22
North Dakota	2		3			5
Oklahoma	13	2	5	2		20
Ohio	168	33	38	9	17	232
Oregon	21	2	10			31
Pennsylvania	112	36	25	8	10	155
Rhode Island	2		4			6
South Carolina	9	2	1			10
South Dakota	6		2			8
Tennessee	14	2	6	2	14	36
Texas	30	8	28		1	59
Utah	11	2	5	1	5	22
Vermont	20	4				20
Virginia	31	1	10	2	5	48
Washington	35	2	11	2	6	54
West Virginia	15	2	3	2		20
Wisconsin	88	8	21	2	16	127
Wyoming	4	2	2		1	7
Canada & Foreign	45	6	310	4	2	361
Total	1534	203	889	85	162	2670

In computing the total circulation in the above table, the new members as listed in the second column, are included in the first column also and consequently are not included in the total column. Ohio leads with 168 members. Ilinois is second with 149, New York third with 142, and Pennsylvania fourth with 112. Pennsylvania leads with thirty-six new members. Ohio is second with thirty-three, Illinois and New York tie for third with seventeen. New York leads with eighty-two subscribers. California is second with forty-eight and Illinois third with forty-seven. Iowa leads in number of student affiliates with sixty-seven. Professor Goss, who is responsible for sending in this large number, is to be congratulated. Iowa has consistently been first in the past years in the number of student affiliates. New York leads in total circulation with 234. Ohio is second with 232 and Illinois third with 203. Pennsylvania is fourth with 155.

Back Copies. During the past year, your Association reproduced 200 copies each of nine separate numbers at a cost of more than $1600. When these Journals are resold, our income is less than half of our cost, but this reprinting of back copies is a service that our Association is doing for the dairy industry. Our income from back copies during the past year amounted to approximately $1500.

Advertising. Our advertising income is on the increase. In 1938 we sold 93.75 pages, and in 1945 we sold 194.5 pages. The following table gives the number of pages of advertising sold for the past eight years:

1938	93.75 pages	1942	143.75 ”
1939	124.00 ”	1943	171.00 ”
1940	126.50 ”	1944	190.00 ”
1941	140.75 ”	1945	194.50 ”

We are pleased to take the opportunity to again express our grateful thanks to the companies that use our pages for advertising. Any courtesies shown our advertisers will be greatly appreciated.

Financial. The income for 1945 amounting to $22,600.42, is the largest income during any year of our organization. Our expenditures which also excelled previous years, were $19,210.22, leaving an operating profit of $3,390.20 as of December 31, 1945. Our net worth was $30,008.17. We had an investment in government bonds of $26,045.00. A complete report of the Certified Public Accountant was sent to each Officer and Director on March 27, 1946.

I wish to take this opportunity to express my gratitude and thanks to the officers and members for the splendid cooperation that they have given me.

Respectfully submitted,
R. B. STOLTZ, *Secretary-Treasurer*

Upon motion duly seconded, the report was approved.

AUDITING COMMITTEE REPORT

The President then requested the report of the Auditing Committee which was given by Mr. O. F. Garrett.

May 27, 1946

To the Directors and Members of the
American Dairy Science Association
Gentlemen:

The Auditing Committee of the American Dairy Science Association met with Mr. Walter C. Burnham, the certified public accountant who examined the financial statement of the Secretary-Treasurer of the American Dairy Science Association. The Committee met with Mr. Burnham in Columbus on May 27, 1946.

Mr. Burnham stated that he had made a careful audit of the accounts, checked the bank statement and reconciled the checking account. He also stated that he counted the bonds held by the Association.

After conferring with Mr. Burnham, the Committee is satisfied that the statement submitted by the Secretary-Treasurer is correct. We recommend that it be accepted by the Board of Directors and the members of the American Dairy Science Association.

Respectfully submitted, IVAN MCKELLIP, O. F. GARRETT, R. A. LARSON

Upon motion duly seconded, the report of the Auditing Committee was accepted and ordered filed.

JOURNAL MANAGEMENT COMMITTEE REPORT

Mr. G. M. Trout, Chairman of the Journal Management Committee, then submitted the following report:

June 17, 1946

To the Executive Board
American Dairy Science Association
Gentlemen:

The Journal Management Committee, A.D.S.A., in session June 17, 1946, submit to the Executive Board for their consideration in rendering greater service to the dairy industry through the JOURNAL OF DAIRY SCIENCE, the following:

1st—Maintain the high scientific standard of the articles covering original work at all times.

2nd—Release abstracts of original articles to the press at the time the page proof of the article is sent to the publisher, the press consisting of trade journals in the various fields.

3rd—Encourage a greater number of good review articles.

4th—Add to the abstract section coverage of patent literature in the field of dairy and allied sciences.

5th—Recast present abstract service looking toward:
  a. More extensive coverage
  b. Better abstracts
6th—Study the possibility of securing and publishing the British Dairy Abstracts.
7th—Furnish membership with more extensive service consisting of book reviews, reviews of new developments in dairy and allied fields, personalia, calendar of coming dairy events, review of outstanding features of such meetings, dairy developments in foreign fields, etc.
8th—Conserve space in the Journal by:
  a. Reducing size of some figures in articles, and by setting small figures at side of page with type around figure.
  b. Reduce size of type in printing abstracts and
  c. Reduce size of margins.

Respectfully,
W. E. Petersen, G. E. Holm,
G. M. Trout, *Chairman*

Inasmuch as the Executive Board did not approve all of these recommendations this report laid over until after the Executive Board made its report. The Executive Board recommended the passage of the first six recommendations and requested that the 7th one be deleted and that No. 8 be studied by the Editor regarding the conserving of space in the Journal and the additional possibility of changing the size of type and making a double column.

Upon motion duly seconded, the recommendations as approved by the Executive Board were voted upon and the motion was lost. It was then moved and seconded that the Journal Management Committee report be adopted. By amendment from the floor, recommendation No. 7 was changed to read "Furnish membership with news of the activities of our members." This amendment was carried. The motion was then put and it was carried.

REPORT OF THE PRODUCTION SECTION

Secretary George H. Wise of the Production Section, presented the following report.

The Production Section held two joint symposia with the Extension Section. The first of these meetings consisted of two phases, one dealing with committee reports and discussions pertaining to breed relations activities and the other with special reports and recommendations of the Dairy Cattle Breeding Committee and of the Pasture and Roughage Committee, respectively. In the second symposium, a panel discussion was presented on the subject of mastitis, including definition, etiology, diagnosis, prophylaxis and therapy.

In order that all research reports might be presented, the Production

Section was divided, thus permitting concurrent sessions. A total of 55 papers were presented. All meetings were well attended.

Two business meetings were held, the first of which was in conjunction with the Extension Section. The following reports were presented, approved and accepted by the two Sections in joint session:

Report of the Pasture and Roughage Committee: This was a summary of the information on the subject of "Evaluation of Pasture in D.H.I.A. Records." The variability in methods of estimating the values of pastures in different states emphasized the need for further study to simplify and to standardize evaluation procedures in various regions and under different systems of management.

The Sections further approved the committee recommendation that the activity of the group be terminated, but that the work on the methods of computing T.D.N. values of pasture be continued by R. E. Hodgson.

The Report of the Dairy Cattle Breeding Committee set forth recommendations for the "Requirements for Recognition as an Artificial Breeding Organization."

The Report of the Dairy Cattle Health Committee reviewed the development and progress of this committee, directed attention to the mastitis symposium this year as a result of their activity and suggested that brucellosis be the subject of discussion in 1947.

The Report of the Committee on Committee Revision recommended "that all transactions between the Purebred Dairy Cattle Association or the Dairy Breed Registry Organizations and the American Dairy Science Association be handled through the Breeds Relations Committee."

It was further recommended "that the Breeds Relations Committee refer such matters as may come within the province of other committees to the proper committee for its consideration and recommended action."

In addition to the foregoing committee reports accepted by the combined sections, the following were approved and accepted by the Production Section in a separate business session:

The Report of the Breeds Relations Committee, joint with the Extension Section, recommended that uniform rules of official testing, as developed by a special committee on this subject, be adopted. The report further indicated that the chairman of the Breeds Relations Committee would continue to serve on the Uniform Score Card Committee of the Purebred Dairy Cattle Association. With reference to the testing report forms the committee arranged for printing and distribution and accepted the responsibility of soliciting suggestions for revision of the H.I.R. report blanks.

The Report of the Feeds Relations Committee, in view of duplication of scope of activities, recommended that the committee be disbanded and that its activities be transferred to those representatives of the American Dairy Science Association serving on the college Feed Advisory Committee to the Feed Industry Council.

The Committee on Resolutions of the Production Section consisted of H. P. Davis, R. E. Hodgson and A. A. Borland, Ch. Their report was accepted and approved.

The Nomination Committee of the Production Section recommend that the incumbents, Vice-Chairman, D. M. Seath, and Secretary, George H. Wise, succeed to Chairman and Vice-Chairman, respectively. Nominations for secretary were presented. L. A. Moore of Beltsville, Maryland, was elected.

Requests for suggestions for improvement of future programs provoked considerable discussion. The section voted the continuance of concurrent sessions within the sectional program whenever the number of subjects rendered it necessary. The lack of time for adequate discussion of the subject matter was the most serious objection to the present program arrangement. It was suggested that consideration be given to plans that may ameliorate this condition.

Respectfully submitted,

G. W. SALISBURY, *Chairman*
D. M. SEATH, *Vice-Chairman*
GEORGE H. WISE, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE EXTENSION SECTION

Secretary E. H. Loveland of the Extension Section reported the following minutes, and upon motion duly seconded, this report was accepted.

At the opening business session held Tuesday, June 18, with Extension Section Chairman Floyd Arnold presiding, the report of the 1944 meeting was summarized and the report of the chairman for 1945 was read by the Secretary, E. H. Loveland, and both were adopted. Chairman Arnold appointed for Resolutions Committee, Perry, Porter and Regan; Nominating Committee, Scheidenhelm, Gearhart and Nystrom; Revision of Committees—Crandall, Loveland and Johnston.

Kendrick reported for the Testing Committee. It was voted that the report be accepted, that the recommendation on supervision and curricula for training for D.H.I.A. supervisors be adopted and that there be further report later on equipment which was later presented and adopted.

At the joint meeting with Production Section Wednesday forenoon the report of the pasture committee by R. B. Becker was adopted and the committee discontinued.

The report of the Dairy Cattle Breeding Committee was made by C. L. Blackman and adopted.

A recommendation was adopted that transactions between the Purebred Dairy Cattle Association and the American Dairy Science Association be handled by the Breeds Relations Committee and material referred by them to the proper committees.

The final business session was held Thursday morning with Chairman Arnold presiding.

Floyd Johnston made a report of the action of a Holstein-Friesian Association Committee on use of D.H.I.A. records by that association. The Extension Section went on record as favoring the use of these records by the breed association but opposed recognition of D.H.I.A. individual records for breed association publicity.

The following reports were adopted as presented:

> Exhibits Committee by A. R. Porter; Farm Management Committee by R. Connolly; Breeds Relations Committee by Floyd Johnston; Dairy Cattle Health Committee by James Linn; 4-H Club Committee by Joe Nageotte; Type Rating by A. C. Baltzer.

Quality Committee by Everett Wallenfeldt. In this last report it was voted that the committee be discontinued but that the Manufacturing Section be asked to include an extension representative on their committee.

A motion was adopted urging all Purebred Dairy Cattle Clubs to send herd test records to the Bureau of Dairy Industry to be included in the proving of bulls.

The Resolutions Committee report was adopted and has been reported to the American Dairy Science Association Committee.

The group adopted amended recommendation on committee revision that standing committees be Breeds Relations; Dairy Cattle Breeding; Dairy Records; Dairy Cattle Health; 4-H Club; Teaching Methods and Program and that special committees be appointed as needed.

The report of the Nominating Committee that Arthur Porter of Iowa be secretary for the ensuing year was unanimously adopted.

The recorded attendance was 119.

Respectfully submitted,

E. H. LOVELAND, *Secretary*

REPORT OF THE MANUFACTURING SECTION

Secretary N. S. Golding of the Manufacturing Section gave the following report and upon motion duly seconded it was accepted.

The programs for the Manufacturing Section were held during the afternoon of Tuesday, June 18, and Wednesday, June 19, and Thursday, June 20, with L. K. Crowe as general chairman. Thirty-four titles were accepted for the program, and only two of the papers (M3, and M20) were not given. In addition to the regular papers, a Quality Symposium was held on Milk, Ice Cream, Butter, Cheese and Manufactured Products, with H. F. Judkins as chairman.

The Business Meetings of the Manufacturing Section were held Wednesday afternoon and Thursday morning. The Standing Committee reports were presented and accepted from the following committees:

(a) Committee for Milk and Cream—K. G. Weckel, Chairman (three of his sub-committees submitted written reports)
(b) Committee on Products Judging—G. M. Trout, Chairman.

Motions were passed as follows:

1. That the Milk and Cream Committee consider the advisability of working to the end of standardization of the Babcock test procedure throughout the country for approval of the Association and that it work with the A.O.A.C. organization.
2. That the chairman appoint a committee to study the matter of permanent records and that this committee also study the organization of the section.
3. That both titles and abstracts be submitted together to the Program Committee for the Annual Meeting.

The two officers elected were Paul R. Elliker, Lafayette, Indiana, Vice-chairman, and J. M. Trimble, Lebanon, Ind., Secy. The vice-chairman of the past year, C. L. Hankinson, Minneapolis, automatically becomes chairman for the coming year.

Respectfully submitted,
N. S. Golding, *Secretary*

NATIONAL RESEARCH COUNCIL REPORT

Mr. C. Y. Cannon who represents our Association on the National Research Council, gave the following report, and upon motion duly seconded the report was accepted.

My appointment as delegate of the American Dairy Science Association to the National Research Council for the years 1945–48 was made by President Nelson last August. Since that time I have tried to become thoroughly acquainted with the work of the National Research Council and the relation the American Dairy Science Association has to it. As you know the Dairy Science Association is allied with the Division of Biology and Agriculture of the Research Council. This Division has 27 affiliated member societies which includes such widely varying organizations as the American Dairy Science Association and the American Society of Ichthyologists and Herpetologists.

Several committees operating under the sponsorship of the Division of Biology and Agriculture are of particular interest to men in the dairy fields. Their recent annual reports indicate their activities. Under the direction of the Agricultural Board an *ad hoc* committee on the production and distribution of milk has been set up to work jointly with the Foods and Nutrition Board to study (1) legislative requirements and restrictions that do not provide an adequate return; (2) regional competition and (3) monopolistic practices that occur in specific milk sheds.

Another committee is also being formed to compile information on the composition of all feed stuffs. A grant of $18,000 from Swift and Company will finance this operation.

During the past year the Committee on Animal Nutrition has issued, among other reports, one entitled "Recommended Nutrient Allowances for Dairy Cattle" which is especially helpful to dairymen.

The American Dairy Science Association requested of the National Research Council, its Interassociation Council and Agricultural Board that a committee be formed to study the public health aspects of brucellosis. Since such a study involves the Division of Medical Sciences, it was finally decided that two committees be appointed, with proper liaison between them, one by the Division of Biology and Agriculture and the other by the Division of Medical Sciences. Although Dr. Frank J. Sladen, Physician and Chief of the Henry Ford Hospital in Detroit, has been appointed as chairman of the committee on the human side, no other appointments to either of the committees have yet been made.

Two other aspects of animal health have been investigated (1) by the subcommittee on mastitis, whose report was published in the March 1946 issue of the Journal of the American Veterinary Medical Association and (2) by the subcommittee on bloat, whose report appeared in the August 1945 issue of the Journal of Animal Science.

Your representative attended the annual meeting of the Division of Biology and Agriculture held in Washington, D. C., on Saturday, April 13, 1946. This meeting listened to the following reports:

Present Status of the National Science Foundation Bill by Dr. Barry Commoner.

Projected Institute of American Biologists by Dr. Detlev W. Bronk.

The Prevention of Deterioration of Equipment in the Tropics by Dr. Glenn A. Greathouse.

The Work of the Insect Control Committee by Dr. R. A. Ormsbee.

Problems of Scientific Personnel by Dr. M. H. Trytten.

In addition, Dr. D. W. Bronk, Chairman of the Committee on International Scientific Relations, reported the activities of his committee in establishing contact with scientists in foreign countries and providing them help where and when needed.

Two requests for action by member societies came out of this meeting. The first request was that backing be given to the A.A.A.S. committee, recently set up for the purpose of supporting Senate Bill 1850 which provides funds for an enlarged research program. This bill is a compromise between the Kilgore and Magnuson bills and has passed the Military Affairs Committee and is reported out to the Senate. Although it is now on the Senate Calendar, Senator Thomas (Utah), Chairman of the Military Affairs Committee, indicated that the bill would probably die there unless very strong backing were given him by scientists to get Senate action. The

American Dairy Science Association should make known their attitude in this matter.

The second matter for action by our association is relative to the establishment of an "Institute of American Biologists" which was proposed and discussed during the April meeting in Washington. Stress was put on the importance of not viewing such a new organization as just another society but to think of it as an operating agency to foster the betterment of biological workers in their political relationships with schools, industry, government and society in general.

Dr. Robert F. Griggs, Chairman of the Division of Biology and Agriculture, requested that a delegate representing the American Dairy Science Association be appointd to participate in a meeting which he will call some time in the near future to establish this institute. Dr. Nelson, our president, has been informed of this matter and will probably follow the desires of the Association members in any action he may take about it.

Respectfully submitted,

C. Y. CANNON

INTER-ASSOCIATION COUNCIL ON ANIMAL DISEASE AND PRODUCTION REPORT

As a result of the cancellation of meetings of some of the member associations during the latter stages of the war, there was little opportunity for the council to hold formal meetings.

Your representative is pleased to report, however, that the U. S. Live Stock Sanitary Association reconsidered the Council's program entitled "Promoting and Protecting the Welfare of the Livestock Industry" at their annual meeting in December, 1945, and gave it their hearty approval. Thus, the program stands approved by all the member organizations. At a meeting of the Council, to be held during the summer of 1946, appropriate steps will be taken to activate those portions of the program which are not currently being handled by other agencies.

It is gratifying to know that as a result of the recognition of the need for vital statistics, and stimulated in part by the council and the committee on Veterinary Services for Farm Animals of the National Research Council, the Iowa Experiment Station and the Agricultural Research Administration of the U. S. Department of Agriculture are conducting a pilot plant study on ways and means of obtaining vital statistics and the evaluation of the economic importance of current losses in production.

Your representative is also pleased to advise that the Agricultural Board of the National Research Council has favorably received the suggestion that a comprehensive study be executed on the relation of brucellosis to public health. It is expected that a report will be issued in due course.

The programs on animal production, which were developed at the request of the Council and distributed by the National Livestock Conservation Program, are being discontinued in view of the fact that the latter organization is being liquidated.

There have been no changes in the membership of the Council during the past year. The financial condition of the Council remains unchanged, with a bank balance of $386.95. The fund has accumulated as a result of the action of the representatives who have refused to accept payment for operating expenses during the previous two years. In view of the fact that a reserve fund has been established, together with other considerations, the Council did not request additional contributions from the member associations for the year October 1, 1945, to October 1, 1946.

Respectfully submitted,

W. E. PETERSEN, *Representative to The Inter-Association Council*

The above report was given by Mr. Petersen and upon motion duly seconded was accepted.

NECROLOGY COMMITTEE

Harry W. Cave of the Necrology Committe read the following report, and upon motion duly seconded, it was adopted.

The Necrology Committee regretfully reports the following members as having passed away during the past year:

Martin Van Scheid was killed in action on Guam, July 29, 1944.

Arnold Lohmann was killed in action in Germany in December, 1944.

Daniel J. Luby died May 24, 1945.

Roscoe W. Clowes, born September 13, 1899, at Stockton, California, died June 5, 1945, at Stockton. He was owner of a dairy business and received a B.S. degree from the University of California in 1923.

Ralph Purdy Gingerich, born January 9, 1888, in Minonk, Illinois, died February 28, 1946, in Los Angeles, California. He was Market Milk Specialist for the California State Department of Agriculture and received a D.V.M. degree from Chicago Veterinary College in 1913.

Stanley M. Ross, 64, President of the M. & R. Dietetic Laboratories, Inc., and one of the founders of the Moores & Ross Company of Columbus, Ohio, died early Friday, March 22, 1946, at his winter home in Miami Beach, Florida. Recognized as one of the leading dairy figures in the United States, Mr. Ross and Harry Moores established Moores & Ross in 1903. In 1929, the company was acquired by the Borden Company and Mr. Ross became Mid-West district chairman of Borden's. He retained this position and also was a director and member of the executive committee of The Borden Company until his retirement because of ill health in 1939. Mr. Ross was president of the International Association of Ice Cream Manufacturers in 1928, was a principal organizer of the Ohio Dairy Products

Association, and was a director of the International Association of Milk Dealers.

Dr. Eraldo T. Bertonasco, 38, a member of the American Dairy Science Association, died at Rochester, Minnesota, on April 8, 1946, of a heart attack. Dr. Bertonasco was Vice-President in Charge of Production of the Stella Cheese Company. He was born in Vesime, Italy, in 1908, the son of Francis and Delfina Bertonasco. He was married in Turin, Italy, March 5, 1932, to Lydia Castigliano, daughter of Attilio Castigliano, President of the Stella Cheese Company.

Clarence B. Frink died July 7, 1945.

Respectfully submitted,
H. W. CAVE
G. A. RICHARDSON
D. R. THEOPHILUS, *Chairman*

RESOLUTIONS COMMITTEE

Mr. H. C. Jackson, Chairman of the Resolutions Committee, submitted the following report, and upon motion duly seconded, it was adopted.

WHEREAS: There is a growing relationship in our intercourse with foreign countries economically, culturally and otherwise, and

WHEREAS: The dairy industry in its various phases of production, processing and manufacturing is sharing in this relationship, and

WHEREAS: Exportation always has its problems in reference to quality and never more so than in the exportation of breeding animals from the standpoint of health, production, pedigree, individuality, adaptability, etc.,

Therefore, in view of the different problems, be it *Resolved:* That the American Dairy Science Association urge unusual care on behalf of purchasers so as to avoid putting the American dairy industry in ill repute in foreign lands.

WHEREAS: Iowa State College, through its administrative staff, its faculty, and especially the members of the Animal Husbandry and Dairy Industry Departments have made available to the American Dairy Science Association in this its 41st Annual Meeting all needed physical facilities for the meeting, and

WHEREAS: Every possible personal courtesy has been given to members of the asociation,

Therefore, be it *Resolved:* That the American Dairy Science Association take this opportunity officially to extend its thanks and appreciation and to request the President of this association to express this appreciation by letter to President Charles E. Friley and to Dean H. H. Kildee.

WHEREAS: The Borden Company has again considered it desirable to offer their award for outstanding research in dairy manufacturing and production, Therefore, be it *Resolved:* That the American Dairy Science Association express to the company its sincere appreciation of this evidence of the continued and great interest in research, which plays such a vital role in the progress of our industry.

WHEREAS: The need is recognized for an effective and uniform milk inspection service, and

WHEREAS: The members of this association train individuals for this specialized service and advise producers and plant operators concerning methods which will result in the efficient production of wholesome milk and dairy products,

Therefore, be it *Resolved:* That the President of this association appoint a committee composed of representatives of the three branches of the association to determine the most effective methods of producing high quality milk and dairy products in the light of present day scientific knowledge.

F. W. ATKESON, E. L. FOUTS, R. V. HUSSONG,
I. R. JONES, H. C. JACKSON, *Chairman*

REGISTRATION COMMITTEE

Mr. E. F. Goss made the following report for the Registration Committee, and upon motion duly seconded, it was accepted.

Illinois	50	S. Dakota	6	Kentucky	3
Iowa	43	Kansas	5	Utah	3
Minnesota	30	Nebraska	5	Georgia	2
Wisconsin	29	Texas	5	Idaho	2
New York	28	Vermont	5	Louisiana	2
Ohio	27	Washington	5	New Hampshire	2
Michigan	19	W. Virginia	5	Oregon	2
Missouri	17	New Jersey	7	So. Carolina	2
Washington, D. C.	14	Connecticut	4	Wyoming	2
Pennsylvania	13	Massachusetts	4	Canada	2
Indiana	11	Montana	4	Arkansas	1
Maryland	10	Oklahoma	4	Maine	1
California	7	Virginia	4	North Dakota	1
Tennessee	7	Colorado	3	New Mexico	1
No. Carolina	6	Florida	3		
					406

Registration of members	406
Registration of non-members	126
Total registration	532
Women and children	225
Total attendance	757

Members from 42 states, the District of Columbia, and Canada were in attendance. Non-members attended from Belgium, China, Denmark and Sweden.

The Secretary then read the minutes of the Executive Board, and upon motion duly seconded, they were adopted. The Secretary then moved and it was seconded by O. F. Hunziker that all action of the Executive Board during the past year be approved.

MEETING OF THE EXECUTIVE BOARD AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. Stoltz, *Secretary-Treasurer*

The Executive Board transacted the following business:

Approved Editor's Report.

Approved Secretary-Treasurer's Report.

Approved and prepared a budget of $23,000 for the year 1947.

Approved the issuing of certificates for student branches of American Dairy Science Association.

Authorized the Secretary to purchase some back numbers of the Journals at $3.00 per volume.

Approved that our Association advise the Pure-bred Cattle Association that we will cooperate with the Dairy Cattle Research Association as is outlined in a letter from Mr. G. A. Bowling of January 8, 1946.

Approved the revised report of the Journal Management Committee as follows:

1st—Maintain the high scientific standard of the articles covering original work at all times.

2nd—Release abstracts or original articles to the press at the time the page proof of the article is sent to the publisher, the press consisting of trade journals in the various fields.

3rd—Encourage a greater number of good review articles.

4th—Add to the abstract section coverage of patent literature in the field of dairy and allied sciences.

5th—Recast present abstracts service looking toward:
  (a) More extensive coverage.
  (b) Better abstracts.

6th—Study the possibility of securing and publishing the British Dairy Abstracts.

7th—That the Editor study the Committee's recommendation concerning the conserving of space in the Journal including the additional possibility of changing the size of type and making a double column.

Approved that these recommendations go into effect on January 1, 1947.

Approved the Inter-Association Council Report.

Accepted the National Research Council Report.

Accepted tentatively an invitation from the University of Illinois to meet in Chicago in 1947.

Employed Secretary-Treasurer for the year 1947.

Made record of the report of nominating committee and results of election.

The following officers were elected:

PAUL H. TRACY, *Vice-president*
C. Y. CANNON, *Director*
S. J. BROWNELL, *Director*

Accepted the resignation of Editor T. S. Sutton and the employment of I. A. Gould as Editor to begin January 1, 1947.

AMERICAN DAIRY SCIENCE ASSOCIATION ANNUAL BANQUET

Ames, Iowa, June 20, 1946

Mr. C. Y. Cannon of Iowa State College was toastmaster at the annual Association banquet and presented President J. A. Nelson who installed the officers elect as follows: Mr. Fordyce Ely of Kentucky was installed as President, Mr. Paul H. Tracy of Illinois as Vice-President, Mr. C. Y. Cannon of Iowa and S. J. Brownell of New York (in absentia) as Directors.

"Mr. Ely, you are about to take over the responsibilities of President of the American Dairy Science Association. As president it will be your duty to be Chairman of the Executive Board and submit to the board for approval the nominations of members to fill vacancies that may occur among the elected officers of the Association. As president you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now charge you with the honor of being President of the American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto."

"Mr. Tracy, you are about to take over the responsibilities of Vice-President of the American Dairy Science Association. As vice-president, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Ely's term, you will automatically become President of this Association. I now charge you with these duties."

"Mr. Cannon and Mr. Brownell (who has been called to Germany), you were elected to the Executive Board of the American Dairy Science Association. It will be your duty to pass on all applications for the establishment of divisions, sections and student branches of the Association. The budget will be under your control. You will have full control of the business of the Association and have title to all property and funds of the Association. You will be members of a Board that has all the rights and powers vested in the by-laws of the Association. With these privileges,

FORDYCE ELY, *President*

responsibilities and obligations you are now considered as members of the Executive Board of the American Dairy Science Association."

PRESENTATION OF ASSOCIATION AWARD

The toastmaster then introduced Mr. H. P. Davis, Chairman of the Association's Honors Committee. Mr. Davis then made the following citation:

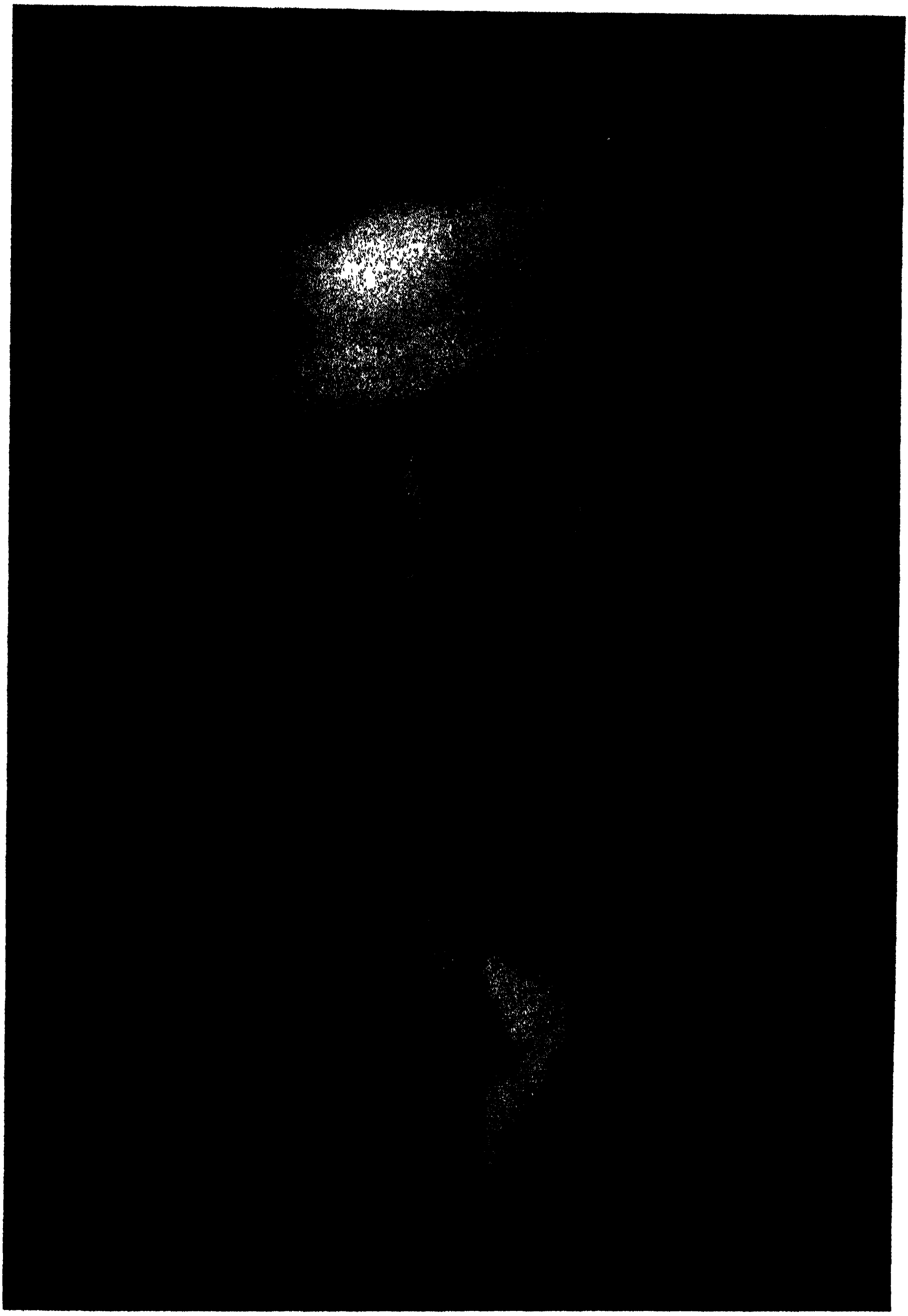
A native of Missouri and educated in the schools of that state, he graduated from the University of Missouri and received also a Masters degree from that institution. Starting his research and teaching at his Alma Mater, he later continued his work at Purdue University before becoming Chief of the Dairy Department of the Kansas State College. Returning later to head the Dairy Department at Purdue, he left to become manager of a large Ayrshire farm. Again he entered the collegiate field as Chief of the Dairy Department of the Michigan State College. In 1928 when a chief for the Bureau of Dairy Industry of the United States Department was needed, he was selected and has filled and continues to fill that important position with ability and wisdom.

During the years his influence has been impressed upon the dairy industry in a multitude of ways. The early work with Eckles on growth of dairy heifers was a milestone and from that time on his energies have always been directed in bringing about more efficient methods for the dairy industry. He valiantly sponsored the Dairy Herd Improvement Association work in this country and made available the records thus obtained for use in selective breeding. Fundamental research has been carried on under his leadership in dairy cattle feeding, breeding, and management. Studies in the processing of dairy products have led to improvements in the manufacture of cheese, butter, ice cream, and powdered milk as well as in the more effective use of dairy by-products.

As a member of the United States delegations to the World's Dairy Congresses held in 1928, 1931 and as chairman in 1933 and 1937, he ably represented this country abroad and did much to familiarize foreign dairy workers with our dairy developments.

O. E. Reed is a member of many important organizations including the National Research Council, the American Association for the Advancement of Science, the American Society of Animal Production, the Dairy Federation, the American Dairy Science Association (past president), Alpha Zeta and Sigma Xi.

It is with a great deal of pleasure that I have the honor to present on behalf of the American Dairy Science Association this award of honor to you, Ollie Ezekiel Reed, an administrator, a scientist, and a teacher in the dairy industry.



OLLIE EZEKIEL REED

PRESENTATION OF BORDEN AWARDS

Mr. W. A. Wentworth of the Borden Company was then introduced and gave the purpose of their sponsoring these awards and introduced the recipients of the Borden Awards in Dairy Manufacturing and Production for the past nine years.

Borden Award in Dairy Manfucturing

Mr. H. F. Judkins, Chairman of the Borden Award Committee for Manufacturing was then introduced and made the following statement.

The recipient of the Borden Award in Dairy Manufacturing for this year is a practical dairy scientist who has made many notable contributions to our knowledge in the field of Dairy Technology, devoting most of his research to the field of market milk, ice cream, condensed milk and dried milk powder. He has been a pioneer in certain aspects related to these fields and he has contributed greatly to our knowledge of the use of the vacuum pan for making ice cream mixes; the study of homogenized milk problems; oxidized flavors in dairy products; the causes of shrinkage in ice cream; the factors affecting the feathering of coffee cream; the sanitary and physical properties of paper milk containers; the use of corn sugar and syrup in dairy products; and the keeping quality of sprayed dried whole milk.

He received his bachelor's, master's and doctor's degree from the University of Illinois in 1920, 1922 and 1930, respectively. It was the unanimous decision of the Borden Award Committee for Dairy Manufacturing that Dr. Paul H. Tracy be selected as the winning candidate.

In addition to his work in research, he has contributed much to the dairy industry, having formed the Chicago Dairy Technology Society in 1930 and has been its secretary since that time. He continues to be one of the leaders in research in dairy manufactures, and at the same time embraces the opportunity of translating the findings of his research to the layman.

From 1920 to 1946, Dr. Tracy has served in the Department of Dairy Husbandry passing from the rank of assistant to Professor, the position which he now holds. He has been active in the affairs of the Association as Secretary of the Manufactures Section in 1933; Chairman of the Manufactures Section in 1937; abstract editor of the JOURNAL OF DAIRY SCIENCE, Director, 1944–46; and Vice-president elect 1946.

Mr. Wentworth then presented to Mr. Tracy a gold medal and $1.000.

Borden Award in Dairy Production

Mr. J. B. Fitch, Acting Chairman of the Borden Award Committee for Production, was then introduced and made the following statement.

The Dairy Production Award Committee has unanimously selected as the

PAUL H. TRACY

winner of the Borden Award for 1946, Dr. Ezra Paul Reineke of Michigan State College.

Dr. Reineke received a B.S. degree with distinction from the University of Minnesota in 1934, majoring in dairy production with emphasis upon agricultural biochemistry. He then went to the University of Missouri for graduate work, receiving his Master's degree in 1936. From 1936 to 1937 he was in charge of the office of extension work of the American Jersey Cattle Club. He returned to the University of Missouri as an instructor in 1938 and received his Ph.D. degree in 1942 when he was promoted to assistant professor. In 1945 he was appointed assistant professor of Physiology and Pharmacology at Michigan State College.

He is author or co-author of 41 scientific publications including four Research Bulletins. These research publications deal with the precursors of the various constituents of milk and more recently with the synthesis of thyroprotein which has been found to favorably influence the secretion of milk in dairy cattle.

In connection with the study of milk precursors and the chemical transformations of blood constituents into milk, Dr. Reineke was the first to present direct evidence that a glycoprotein fraction of the blood was concerned in the synthesis of milk protein. As a result of studies on the mammary gland respiratory quotient of animals under various conditions, evidence was presented that suggest the synthesis of short-chain fatty acids by the mammary gland from carbohydrate precursors.

During the past five years his research has centered around the problem of determining the chemical conditions favoring the transformation of casein into synthetic thyroprotein. The initial iodinated casein preparations were about equal to desiccated thyroid in thyroidal activity. As a result of his studies, methods were developed which increased the thyroid activity of thyroprotein fifty times. It is interesting in this connection to note that although intensive study was given to the problem of thyroprotein synthesis in England during the War years, they reported in 1945 that Reineke's methods had not been improved upon. At least one-third of the total tyrosine in casein has been converted to thyroxine by his methods.

Thyroprotein is the first hormone substance which bids fair to earn for itself an important place not only in the dairy industry in stimulating increased milk and fat production when administered in the dairy ration, but in all branches of the livestock and poultry industries. Preliminary observations with thyroprotein indicate that this product may also become of importance in problems of sterility of livestock.

One of the important problems in the use of thyroprotein is the problem of standardization. Dr. Reineke developed a chemical method for the determination of the thyroxine content as well as several biological methods which will aid in producing a uniform product.

E. P. REINEKE

Dr. Reineke is not only a brilliant scientist but by birth and experience, he is a practical dairyman. He was born and raised on a dairy farm. He earned his college expenses by caring for the University experimental herd. He was in charge of the office extension work of a national breed association.

Dr. Reineke has those unusual and outstanding qualifications for a recipient of the Borden Award. He is young, has a brilliant record of scientific contributions and a background of practical experience and application. We believe that in the years ahead of him, he will add many and even greater contributions to dairy science.

Mr. Wentworth then presented to Dr. Reineke a gold medal and $1,000.

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FACTORS INFLUENCING ACID PRODUCTION BY CHEESE CULTURES. I. EFFECT OF COOKING TEMPERATURES ON ACID PRODUCTION IN THE MANUFACTURE OF CHEDDAR CHEESE[1]

F. J. BABEL

Iowa Agricultural Experiment Station, Ames, Iowa

The rate of lactic acid development by cheese cultures has received considerable attention in the past few years. Increased interest in acid development appears to be due to the larger amount of cheese being manufactured from pasteurized milk and the adoption of a definite time-schedule in the manufacturing operation. The schedules which have been proposed assume that a certain degree of acidity will be attained in a certain period of time. In order to follow such a method as closely as possible, it is of paramount importance that the cheesemaker have a culture that will produce acid uniformly and consistently from day to day. The cheesemaker should also be familiar with the particular culture he is using and know certain of its limitations.

In the manufacture of both semi-commercial and experimental lots of cheese in the cheese laboratories of Iowa State College, many cheese cultures have been studied with regard to rate and constancy of acid production. During the course of these trials, several instances of slow acid production occurred and these were studied in detail. Slow acid production was attributed to two factors: (a) the cooking temperature employed, and (b) the presence of bacteriophage. These two factors will be discussed separately.

HISTORICAL

The influence of temperature on acid production by cheese cultures has been studied by several investigators. Whitehead and Cox (6) noted that strains of lactic acid streptococci which could withstand temperatures of 37° C. (98.6° F.) developed acid in a normal manner right up to the salting stage in the manufacture of cheddar cheese when cooking temperatures of 37° to 39° C. (98.6° to 102.2° F.) were employed. Strains that could not

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withstand a temperature of 37° C. (98.6° F.) produced acid normally during the early part of the cooking period, and the manufacturing process appeared to proceed normally until the whey was drained; after the whey was drained slow acid production persisted throughout the remainder of the manufacturing process.

Harrison and Dearden (4) determined the rate of acid production by three cultures at temperatures of 22°, 28°, 30°, 37°, 40° and 45° C. (71.6°, 82.4°, 86°, 98.6°, 104° and 113° F.). Culture S produced acid at temperatures up to 37° C. (98.6° F.) but failed to produce acid at 40° C. (104° F.). Culture X as well as culture Y produced acid at 40° C. (104° F.). All of the cultures failed to grow at 45° C. (113° F.). After incubation at 40° C. (104° F.) for 24 hours, culture S was incapable of further growth at either 22° or 28° C. (71.6° or 82.4° F.). Cultures X and Y were capable of growth at the lower temperatures after 24 hours incubation at 40° C. (104° F.).

Dolby (2) studied the effect of cooking temperatures of 100° and 104° F. on the rate of acid formation in the manufacture of cheddar cheese; one culture was employed in these trials. With the higher cooking temperature, growth of the culture was restrained to such an extent that acid development in the later stages of the manufacturing process was definitely retarded. The pH of the young cheese cooked to the high temperature was considerably higher than that cooked at the lower temperature.

Golding *et al.* (3) concluded that a cooking temperature of 102° F. greatly retarded the development of acidity and the longer the culture organisms were held at this temperature, the slower was their subsequent development of acidity when returned to 86° F. These investigations were carried out in milk and not in actual cheesemaking operations.

Dahlberg and Ferris (1) inoculated milk with a cheese culture and held one portion at 86° F. and another portion at 100° F. Acid development at 86° F. was rapid, while at 100° F. it was very slow. When the milk was held at 86° F. for 2 hours followed by incubation at 100° F., acid development was good. In actual cheesemaking operations, when bacterial multiplication of cultures was commenced at 86° F. before cooking to 100° F., the acid developed satisfactorily at 100° F.

METHODS

Source of cultures. The cultures used in these studies came from the following sources: Cultures 1 and 5 were from the regular collection maintained in the Dairy Industry Department, Iowa State College. Cultures 2, 6 and 7 were obtained from commercial sources. Cultures 3 and 4 were prepared for experimental purposes; the organisms were obtained from sour cream.

Propagation of cultures. Whole mixed milk, heated to 200° F. for 1 hour, was used for propagation of the cultures. After heating, the milk was cooled to 70° F., inoculated with approximately 1 per cent culture, and incubated at 70° F. for 16 hours. After the incubation period, the cultures were placed in a refrigerator at a temperature of 34° F. The cultures were transferred every day except Sunday and had been transferred in this manner several months before this work was begun.

Titrable acidity determinations. The acidity determinations were made by titrating a 9-gram sample with N/10 sodium hydroxide, using phenolphthalein (5 drops of a 1 per cent solution in 50 per cent alcohol) as the indicator.

The rate of acid production by cultures in skim milk was determined on various days. The skim milk used in these trials was prepared from one lot of skim milk powder; 90 grams of powder were dissolved in 1 liter of distilled water. The reconstituted skim milk was heated to 180° F. for 10 minutes, cooled to the desired temperature, inoculated with culture and divided into portions of 250 ml.

The temperature of the cultures held at 86° and 98° F. was maintained by use of a water bath placed inside a thermostatically controlled incubator. The temperature of the cultures held at 101° and 104° F. were controlled by means of water baths; the variation in temperature was ± 0.5° F.

Manufacture of cheese. The method followed for manufacturing the cheddar cheese was essentially that outlined by Price (5) except that the curd was milled at a whey acidity of 0.50 per cent, calculated as lactic acid.

RESULTS

The experimental work was designed to demonstrate: (a) the effect of temperature on acid production by cheese cultures when the cultures were inoculated into skim milk and held for the entire incubation period at temperatures of 86°, 98°, 101° and 104° F., (b) the effect of a short exposure to a temperature of 104° F. on acid production by cheese cultures growing in skim milk, and (c) the effect of cooking temperatures of 100°, 102° and 104° F. on acid development in the manufacture of cheddar cheese.

Table 1 presents the data on rates of acid production as measured by the increases in titrable acidity, at temperatures of 86°, 98°, 101° and 104° F. Cultures 2, 5 and 6 produced acid more rapidly at 86° F., while cultures 1, 3, 4 and 7 produced acid more rapidly at 98° F. All of the cultures produced acid slowly at temperatures of 101° and 104° F. There was very little difference in the amounts of acid produced during the first two hours of incubation, regardless of the temperature employed. Although very little acid was produced by the cultures at 101° and 104° F., there were differences in acid production at these temperatures. Cultures 1, 5 and 6 produced practically no acid after being held at 101° or 104° F. for 4 hours. Cultures 2, 3, 4 and 7 showed some increase in acidity at 101° F. throughout the holding period and cultures 4 and 7 showed some increase in acidity at 104° F.

The rates of acid production by cultures held (a) continuously at 86° F., and (b) for 2 hours at 86° F., then 2 hours at 104° F., and then 3 hours at 86° F. are given in table 2. Culture 3 was the only culture that produced acid more rapidly when returned to 86° F. after being held at 104° F. for 2 hours than when held for the entire period at 86° F. Culture 4 was inhibited slightly by the heat treatment. Immediately after the heating period, or after 4 hours, the cultures receiving the heat treatment had produced larger amounts of acid than the cultures held continuously at 86° F.; subsequent incubation, however, showed that the heat treatment decreased final acid production with all except culture 3. The various cultures showed appreciable differences in rates of acid production.

TABLE 1

Rates of acid production by cheese cultures when held at various temperatures

Culture No.*	Temperature of incubation (°F.)	Titrable acidity (ml. N/10 NaOH per 9 grams)					
		0 hours	2 hours	4 hours	5 hours	6 hours	7 hours
1	86	1.35	1.60	2.10	2.45	2.90	4.00
	98	1.35	1.65	2.30	2.80	3.50	4.30
	101	1.35	1.60	1.65	1.70	1.65	1.65
	104	1.35	1.55	1.55	1.60	1.65	1.65
2	86	1.40	1.50	2.05	2.65	3.85	5.50
	98	1.40	1.60	2.02	2.85	3.85	4.85
	101	1.40	1.60	1.85	2.00	2.20	2.30
	104	1.40	1.60	1.70	1.75	1.80	1.85
3	86	1.45	1.55	2.05	2.40	3.00	4.35
	98	1.45	1.60	2.15	2.50	3.40	4.55
	101	1.45	1.55	1.75	1.80	1.85	1.95
	104	1.45	1.50	1.65	1.70	1.70	1.85
4	86	1.40	1.55	1.80	2.25	3.05	4.00
	98	1.40	1.50	2.10	2.85	4.05	4.75
	101	1.40	1.50	1.80	2.05	2.30	2.60
	104	1.40	1.55	1.75	1.85	2.00	2.15
5	86	1.45	1.60	2.05	2.40	3.10	4.35
	98	1.45	1.70	2.25	2.55	2.90	3.25
	101	1.45	1.65	1.70	1.75	1.80	1.80
	104	1.45	1.65	1.65	1.65	1.65	1.70
6	86	1.40	1.50	1.90	2.20	2.55	3.10
	98	1.40	1.55	1.85	2.00	2.20	2.40
	101	1.40	1.50	1.70	1.70	1.75	1.75
	104	1.40	1.50	1.60	1.65	1.65	1.75
7	86	1.40	1.60	2.00	2.40	2.80	3.30
	98	1.40	1.65	2.15	2.65	3.15	4.00
	101	1.40	1.60	1.90	2.05	2.20	2.35
	104	1.40	1.60	1.85	1.95	2.10	2.20

* A one per cent inoculation was used.

The results of 14 trials involving the use of cooking temperatures of 100°, 102° and 104° F. in the manufacture of cheddar cheese are presented in

TABLE 2

Rates of acid production by cheese cultures when held (a) continuously at 86° F. and (b) 2 hours at 86° F., then 2 hours at 104° F., and then 3 hours at 86° F.

Culture No.*		Titrable acidity (ml. N/10 NaOH per 9 grams)						
		0 hours	1 hour	2 hours	4 hours	5 hours	6 hours	7 hours
1	a	1.35	1.45	1.60	2.15	2.60	3.40	4.75
	b	1.35	1.45	1.60	2.30	2.60	3.05	3.65
2	a	1.45	1.60	1.80	3.00	4.40	5.90	6.35
	b	1.45	1.60	1.80	3.00	3.65	4.40	5.35
3	a	1.40	1.50	1.65	2.35	2.80	3.60	4.95
	b	1.40	1.50	1.65	2.60	3.15	4.00	5.15
4	a	1.45	1.55	1.75	2.55	3.45	4.70	5.80
	b	1.45	1.55	1.75	2.75	3.30	4.30	5.45
5	a	1.45	1.55	1.75	2.40	3.00	3.85	4.95
	b	1.45	1.55	1.75	2.65	3.15	3.65	4.15
6	a	1.45	1.55	1.75	2.30	2.80	3.60	5.10
	b	1.45	1.55	1.75	2.40	2.70	3.10	4.10
7	a	1.50	1.65	1.80	2.50	2.90	3.40	3.95
	b	1.50	1.65	1.80	2.50	2.60	2.80	3.00

* A one per cent inoculation was used.

table 3. With 5 of the 7 cultures employed, acid production was more rapid at 100° F. than at 102° F.; one culture produced the same amount of acid at both temperatures and one culture produced acid slightly faster at 102° F. A cooking temperature of 104° F. resulted in decreased rates of acid production with all of the cultures as compared with cooking temperatures of 100° and 102° F. The cultures showed variations in the time required to reach a whey acidity of 0.50 per cent. The variation between cultures at 100° F. was 40 min., at 102° F. it was 68 min., and at 104° F. it was 80 min. The average increase in manufacturing time of cheese cooked to 102° F. as compared with a cooking temperature of 100° F. was 14.5 min. for all cultures. When a cooking temperature of 104° F. was employed the time of manufacture was increased 35 min. as compared with the time of manufacture when a cooking temperature of 100° F. was employed. It was noted that when a

TABLE 3

Time required from setting to milling to reach a whey acidity of 0.50 per cent when cooking temperatures of 100°, 102° and 104° F. were employed

Culture No.	Cooking temperature			
	100° F.	102° F.	104° F.	104° F.*
1	4′30″	4′57″	5′15″	4′25″
2	4′10″	4′30″	4′35″	4′02″
3	4′20″	4′10″	4′25″	4′20″
4	4′35″	4′35″	5′00″	4′30″
5	4′45″	5′05″	5′25″	4′50″
6	4′20″	4′37″	5′10″	4′15″
7	4′50″	5′18″	5′45″	4′45″

* Ripened an additional 30 minutes before setting.

cooking temperature of 104° F. was employed, the rate of acid production was less affected if the cultures were growing rather rapidly at the time of cooking. When the vat to be heated to 104° F. was given an additional ripening time of 30 minutes before setting, the time from setting to milling was approximately the same for vats cooked to 100° F. as for vats cooked to 104° F.; the vats being milled at the same acidities.

DISCUSSION

In the several instances prior to the time at which these experiments were begun, in which acid production was slow during the manufacture of cheddar cheese, the slowness was usually first evident at the time of draining the whey or shortly thereafter. In all instances, the cultures appeared to be functioning normally up to this time. Since the slowness occurred right after the curd had been cooked, it appeared probable that the culture being employed might be inactivated by the cooking temperature. For this reason the influence of temperature on acid production by cheese cultures was studied.

Acid production by all of the cultures was fairly rapid when grown in skim milk at temperatures of 86° and 98° F. With some cultures a temperature of 98° F. tended to slightly decrease the rate of acid production in comparison to that produced at 86° F. while with other cultures this temperature appeared slightly more favorable than 86° F. Temperatures of 101° and 104° F. resulted in greatly decreased acid production when the cultures were continuously held at these temperatures during the entire incubation period. In most instances, only slightly more acid was produced at 101° than at 104° F.

The failure of a culture to produce acid when held continuously at temperatures of 101° or 104° F. does not necessarily mean that the culture will fail to produce acid when cooking temperatures of 101° or 104° F. are employed in the manufacture of cheddar cheese. This has been shown by experiments in which the cultures were heated to 104° F. and held at that temperature for 2 hours, and also by experiments in the manufacture of cheddar cheese in which various cooking temperatures were employed. In trials with various cultures that were grown in skim milk and first given an incubation period at 86°.F. before heating to 104° F. the heating resulted in decreased acid production with 6 of the 7 cultures tested; one culture produced more acid. The extent to which acid production was decreased by heating to 104° F. for 2 hours varied with the different cultures, some being affected more than others. In no case did the production of acid nearly cease as a result of the heat treatment.

The cooking temperatures generally used in the cheese laboratories of Iowa State College range from 100° F. to 102° F. However, in the manufacture of experimental lots of cheese in small vats (50 gal.), the temperature at times will accidentally reach 103° or 104° F. Also, variations of 1° or 2° F. are quite common with certain dairy thermometers. When cooking temperatures of 100°, 102° and 104° F. were employed in actual cheesemaking operations, acid production was most rapid at 100° F. with the majority of the cultures. The results indicate that 102° F. is the maximum temperature which should be used with the majority of cultures. If a higher temperature is desirable, the culture should be checked for its ability to produce acid at the higher temperature and for its ability to produce acid following the heat treatment. When a cooking temperature of 104° F. was employed, it was found to be more time-saving to ripen the milk for a longer period before setting than to ripen for the normal period of time and wait for acid development to occur while cheddaring. Even when the cheese was made by the regular procedure and cooked to 104° F., acid continued to develop, although at a slower rate. No evidence was apparent that temperatures of 104° F. would entirely stop the acid production, or very nearly stop acid production. With certain lots of cheese made prior to these investigations the acid formation practically ceased after the whey was drained following the cooking of the curd.

In order to eliminate the possibility that bacteriophage might account for some of the slow acid production in the trials reported in this paper, the cultures were examined for bacteriophage. In the studies involving the manufacture of cheese, both the culture and whey were examined. Since no phage was evident, the decrease in acid production was solely attributed to the temperature employed.

SUMMARY

The rates of acid production of 7 cheese cultures held at temperatures of 86°, 98°, 101° and 104° F. were determined. Three cultures produced acid more rapidly at 86° F., while 4 cultures produced acid more rapidly at 98° F. All of the cultures produced acid slowly at temperatures of 101° and 104° F.

The rates of acid production of 7 cheese cultures held continuously for 7 hours at 86° F., were compared with those of the same cultures held 2 hours at 86° F., then 2 hours at 104° F., and then 3 hours at 86° F. Six of the 7 cultures produced less acid as a result of the higher temperature and 1 culture produced slightly more acid.

In actual cheese-making operations, a cooking temperature of 102° F. slightly retarded acid development with 5 of the 7 cultures when compared with a cooking temperature of 100° F. A cooking temperature of 104° F appreciably retarded acid production as compared with a cooking temperature of 100° F.

Ripening the milk for a longer period before setting appears to be more time-saving in the manufacture of cheddar cheese when cooking temperatures of 104° F. are employed than does following the regular procedure and waiting for acid development just prior to milling.

A slightly high cooking temperature (104° F.) may be an explanation for somewhat retarded acid development in the manufacture of cheddar cheese but it does not explain cases of greatly decreased acid production or the actual stopping of acid production by cheese cultures.

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FACTORS INFLUENCING ACID PRODUCTION BY CHEESE CULTURES. II. INFLUENCE OF BACTERIOPHAGE ON ACID PRODUCTION IN THE MANUFACTURE OF CHEDDAR AND COTTAGE CHEESE[1]

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Slow acid production by cheese cultures is much more evident in the manufacture of cheddar and cottage cheese than it is in the manufacture of certain other cheeses in which relatively small amounts of acid are developed. In the manufacture of cheddar cheese the acidity is carefully followed for a period of 5 or 6 hours and with cottage cheese, acid development may take place for a period of 14 to 16 hrs. if overnight setting is practiced. In the manufacture of cheeses in which only a small amount of acid is developed, and where the whey may be diluted with water in the manufacturing procedure, it is sometimes difficult to determine whether or not the cheese culture is producing acid properly.

Very little difficulty was experienced with slow acid production in the cheese laboratories of Iowa State College prior to November 1944. At this time considerable difficulty was encountered and the principal cause of this difficulty was bacteriophage. The characteristics of these incidences and results of studies on the causative factor will be presented in this paper.

HISTORICAL

Bacteriophage active against *Streptococcus lactis* has been reported by various investigators in the United States and elsewhere (2, 6, 7, 12). The ability of bacteriophage to influence acid development during cheesemaking has also been reported by various investigators in New Zealand (11), England (1), Australia (10), France (5), Canada (4) and perhaps other countries. Although bacteriophage active against the lactic acid streptococci of butter cultures has been reported in the United States (7), the literature reveals no instance of slow acid production in the manufacture of cheese being directly attributable to bacteriophage.

Whitehead and Cox (12) isolated a bacteriophage from an aerated culture which suddenly failed to produce acid. They emphasized the importance of bacteriophage action in connection with the problem of vitality in cheese cultures.

Bacteriophage was demonstrated in a sample of whey by Sutton (10). When the whey was tested by means of the plate-smear technique it exhibited

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no ability to cause plaque formation but by an enrichment procedure, bacteriophage was produced in demonstrable quantities.

Single-strain cultures of streptococci, used as "starters" in cheddar cheese manufacture in New Zealand, frequently failed to produce acid due to the apparently spontaneous appearance of bacteriophage (13).

The sudden stoppage of acid development in several experimental vats of cheddar cheese was shown by Johns and Katznelson (4) to be due to the activity of a polyvalent streptococcal bacteriophage. Although the culture used consisted of a mixture of organisms, the stoppage was as abrupt as in cases where single-strain cultures were employed. The presence of bacteriophage could not be demonstrated in the culture employed, nor could it be demonstrated in the milk, stable and laboratory air, or rennet extract.

Whitehead and Hunter (14) noted that bacteriophages active against lactic streptococci occurred in the atmosphere of commercial cheese factories. Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for the air-borne bacteriophages. The concentration of air-borne bacteriophages was sometimes so great that it was impossible to prevent infection of the cultures for more than a few propagations. Protection of the cultures from air-borne bacteriophages eliminated culture failures.

Anderson and Meanwell (1) state that culture troubles in the past may have been masked by the use of raw milk for cheese manufacture. The raw milk may have enabled sufficient acid to be produced from the natural flora in spite of partial culture failure, but the quality of the resulting cheese was often variable. Slow acid production was evident only when the culture used was infected with bacteriophage. When the milk used for preparing the bulk culture was slightly infected with bacteriophage, a one thousandfold increase in the amount of bacteriophage took place after over-night incubation in the presence of the susceptible culture. Cultures containing several strains of lactic acid streptococci failed to produce acid the same as single-strain cultures.

In England, Nichols and Wolf (8) found that bacteriophage was most prevalent in May and August. Phaging of cultures was widespread geographically. Studies made on the number of phaging samples obtained from factories making cheese from raw and heat-treated milk showed practically identical results for both raw and pasteurized milk cheese (23 per cent).

Hunter (3) states that the primary source of bacteriophage in cheese factories is the whey. Whey obtained from vats during the manufacture of cheese when acid production was normal frequently showed bacteriophage titers varying from 10^{-1} to 10^{-8}. When a bacteriophage preparation having a titer of 10^{-12} was added to milk in the proportion of 1 : 900,000 to 1 : 700,000, these additions were just on the borderline of causing a marked influence in acid production by the culture.

METHODS

Manufacture of cheese. The cheddar cheese was manufactured from pasteurized milk according to the method of Price (9) with the exception that the curd was milled at a whey acidity of 0.50 per cent when acid production was normal. When acid production was slow, the method of Price (9) was followed until the time of draining the whey. The curd from vats in which acid production was slow, was generally cheddared for a longer period and milled at much lower acidities (0.15 to 0.25 per cent).

The cottage cheese was manufactured from skim milk which had been pasteurized at 145° F. for 30 minutes. The skim milk was set at 72° F. with 1 per cent culture and 1 ml. rennet extract per 1000 pounds. The curd was cut with 0.5-inch knives after the whey acidity had reached 0.5 per cent. It was cooked to the desired firmness by the addition of hot water.

Preparation of bacteria-free filtrates. Bacteria-free filtrates of cheese cultures were prepared by filtering the cultures under aseptic conditions through coarse filter paper and the filtrate thus obtained through a Selas micro-porous filter (#03 porosity).

Bacteria-free filtrates of skim milk and whole milk were prepared by adding sterile lactic acid (10 per cent) in amounts just sufficient to coagulate the milk and then filtering in the same manner as for the cheese cultures.

Bacteria-free filtrates of whey and rennet were prepared by filtering directly through the Selas micro-porous filter.

Isolation of Streptococcus lactis from cheese cultures. Cultures of *S. lactis* were isolated from cheese cultures by plating on tomato agar and picking colonies. The plates were incubated at room temperature for 2 days. The tomato agar was prepared as follows: Ten grams of Bacto peptone, 10 g. of Bacto peptonized milk and 15 g. of agar were dissolved in 600 ml. water by boiling. Then 400 ml. of tomato juice (obtained by filtering canned tomatoes) neutralized to pH 7.0 with N/1 NaOH was added. The medium was filtered, placed in bottles, and autoclaved at 15 lbs. pressure for 25 min.

Formation of bacteriophage plaques. The method used to demonstrate plaque formation was as follows: About 15 ml. of tomato agar was poured into a sterile Petri dish and allowed to harden. A heavy suspension of a sensitive organism was spread over the surface of the agar by means of a bent glass-rod. Then a small amount of the bacteria-free filtrate to be tested for bacteriophage was spread over the surface of the agar and the plate incubated for 16 to 24 hrs. at 30° C.

Sensitivity test. In order to determine whether or not a cheese culture was sensitive to the action of a particular bacteriophage strain, the cheese culture was plated on tomato agar and representative cultures of *S. lactis* picked into litmus milk. The *S. lactis* cultures were transferred into duplicate tubes of litmus milk. One set of cultures served as controls, while the cultures in the other set were inoculated with a drop of the bacteria-free filtrate containing bacteriophage. Retardation of the changes observed in litmus milk (acid, reduction, coagulation) served as the basis for determining whether or not a culture was sensitive. This same procedure was used to detect the presence of bacteriophage in unknown samples; using *S. lactis* cultures of known bacteriophage sensitivity as test organisms.

Bacteria counts. Bacteria counts were made by the plate method, using tomato agar and an incubation of 48 hrs. at 30° C.

Bacteriophage titer. The bacteriophage titer of a bacteria-free filtrate was determined by the dilution method and was recorded as the smallest amount of filtrate, expressed in milliliters, which would cause a significant retardation of acid production, reduction or coagulation of litmus milk.

RESULTS

Effect of bacteriophage on acid production during the manufacture of cheddar cheese. The effect of bacteriophage on acid production in the manufacture of cheddar cheese is presented in table 1. In the trial in which bacteriophage was absent, acid production proceeded normally and the curd was milled at a whey acidity of 0.52 per cent, calculated as lactic acid, 4 hrs. 35 min. after setting with rennet. In this trial the milk, culture and whey were free of bacteriophage in 1-ml. quantities, the largest amount used for detection of bacteriophage. In the trial in which bacteriophage was present, acid production appeared to proceed normally until the time of draining the whey. After the whey was drained, acid production practically ceased. The curd was milled at a whey acidity of 0.17 per cent, 6 hrs. 50 min. after setting with rennet. In this trial the milk was free of bacteriophage when 1-ml. quantities were tested but the culture employed was slightly contaminated with bacteriophage and had a bacteriophage titer of 10^{2}. The whey at the time of draining had a bacteriophage titer of 10^{6} and at the time of milling a titer of 10^{-8}. The mother culture employed in this trial showed no evidence of bacteriophage at the time it was used to inoculate the bulk culture in the cheese laboratory. The bulk culture had coagulated in a normal manner and after 14 hrs. incubation at 70° F., had devel-

TABLE 1

Effect of bacteriophage on acid production in the manufacture of cheddar cheese

Stage of manufacture	Bacteriophage absent		Bacteriophage present	
	Time	Titrable acidity	Time	Titrable acidity
		per cent		*per cent*
Milk (pasteurized)	8:00	0.16	8:00	0.16
Milk plus 1% culture	8:10	0.165	8:10	0.165
Setting	9:10	0.175	9:10	0.175
Cutting curd	9:40	0.12	9:40	0.12
Draining whey	11:10	0.14	11:10	0.13
Milling curd	1:45	0.52	4:00	0.17

oped an acidity of 0.82 per cent. The same mother culture was used to inoculate the bulk culture in both trials.

The effect of bacteriophage on acid production during the manufacture of cheddar cheese was so similar in all trials that the data for only one of the trials are given. Eight other trials showed practically identical results even though different cheese cultures were used. Cheese cultures containing several strains of *S. lactis* (strains showing differences in bacteriophage sensitivity) and also single-strain cultures were employed. In 5 of the 8 other trials bacteriophage could be detected in the bulk culture in small amounts; it could not be detected in the bulk culture in 3 trials but was present in fairly large amounts in the whey. However, acid production practically ceased after the whey was drained in all of the trials.

The ability of the bacteria-free filtrate, prepared from the whey obtained at time of milling (trial reported in table 1—bacteriophage present), to delay acid production by pure cultures of *S. lactis* is shown in table 2. The cultures of *S. lactis* were obtained from the mother culture which was used to inoculate the batch culture employed in the manufacture of the cheese from which the whey filtrate was obtained. The *S. lactis* cultures were picked at random from colonies appearing on a Petri dish. All of the control cultures (litmus milk inoculated with the various strains of *S. lactis*) showed acid, reduction and coagulation after being held for 16 hrs. at 30° C. When parallel cultures were inoculated with a drop of the whey filtrate containing bacteriophage, only 3 of 15 cultures were not sensitive to the action of bacteriophage present in the whey filtrate. After 40 hrs. at 30° C., 6 of the 15 cultures showed acid, reduction and coagulation of litmus milk and after 264 hrs., 7 of the 15 cultures showed acid, reduction and coagulation. There was no evidence of growth in 8 of the 15 cultures. Since the *S. lactis* cultures in this trial had different bacteriophage sensitivities, the cheese culture from which the *S. lactis* cultures were obtained would be considered a multiple-strain culture.

Bacteria-free filtrates of whey were prepared also from vats in which single-strain cheese cultures were employed and in which slow acid production occurred. When *S. lactis* cultures were isolated from these single-strain cheese cultures and tested for bacteriophage sensitivity, using whey filtrates from vats in which the particular single-strain cheese culture was employed, all of the *S. lactis* cultures were sensitive.

All of the bacteria-free whey filtrates (prepared from vats in which slow acid production occurred) which showed indication of the presence of bacteriophage by the sensitivity test were also tested for the presence of bacteriophage by the plaque method. *S. lactis* cultures which exhibited bacteriophage sensitivity according to the sensitivity test also produced bacteriophage plaques when the same bacteria-free filtrate was employed in both instances.

Effect of bacteriophage on acid production during the manufacture of cottage cheese. Data showing the effect of bacteriophage on acid production by a cheese culture in the manufacture of cottage cheese are presented in table 3. At the time of setting, or immediately after the addition of rennet and culture, the skim milk had a titrable acidity of 0.16 per cent and a total bacteria count of 2,460,000 per ml. A bacteria-free filtrate prepared from the vat contents at time of setting showed a bacteriophage titer of 10^{1}. Individual samples of skim milk, culture and rennet extract showed no bacteriophage activity when 1-ml. samples were tested. One hr. after setting the titrable acidity increased to 0.17 per cent and the bacteria count increased to 4,200,000 per ml.; at the same time the bacteriophage titer increased to 10^{2}. Two hrs. after setting the titrable acidity had increased to 0.18 per cent, the bacteria count to 11,800,000 and the bacteriophage titer to 10^{-5}. After 4 hrs., the titrable acidity had increased to 0.20 per cent but the bacteria count showed an appreciable decrease (3,600,000 per ml.); the bacteriophage titer increased to 10^{-7}. Eight hrs. after setting the titrable acidity was 0.21 per cent and the bacteria count had decreased further to 7,300 per ml.; the bacteriophage titer apparently reached its maximum at this time (10^{-9}). There was no increase in the titrable acidity or bacteriophage titer during the period 8 to 16 hrs. after setting but the bacteria count increased to 12,300 per ml. After 16 hrs. both the titrable acidity and bacteria count showed marked increases and the bacteriophage titer remained practically constant. At the 48-hr. period the titrable acidity was 0.68 per cent and the bacteria count 660,000,000 per ml.

TABLE 2

Influence of a whey filtrate containing bacteriophage on growth and acid production by cultures of Streptococcus lactis in litmus milk

Streptococcus lactis culture No.	Control cultures after 16 hrs. at 30° C.	Cultures inoculated with one drop of whey filtrate containing bacteriophage and incubated at 30° C. for				
		16 hrs.	40 hrs.	88 hrs.	136 hrs.	264 hrs.
1	a r c	0	0	0	0	0
2	a r c	0	a r c	a r c	a r c	a r c
3	a r c	0	a r c	a r c	a r c	a r c
4	a r c	0	0	0	0	0
5	a r c	0	0	0	0	0
6	a r c	0	0	0	0	0
7	a r c	a r c	a r c	a r c	a r c	a r c
8	a r c	a r c	a r c	a r c	a r c	a r c
9	a r c	0	0	0	0	0
10	a r c	r	a r c	a r c	a r c	a r c
11	a r c	0	0	0	0	0
12	a r c	0	0	0	0	0
13	a r c	0	0	0	a r c	a r c
14	a r c	0	0	0	0	0
15	a r c	a r c	a r c	a r c	a r c	a r c

a = acid; r = reduction of litmus; c = coagulation; 0 = no evidence of growth.

TABLE 3

Effect of bacteriophage on acid production by a cheese culture during the manufacture of cottage cheese

Time after setting (hours)	Titrable acidity (per cent)	Number of bacteria per ml.	Bacteriophage titer
Initial	0.16	2,460,000	10^{-1}
1	0.17	4,200,000	10^{-2}
2	0.18	11,800,000	10^{-5}
4	0.20	3,600,000	10^{-7}
8	0.21	7,300	10^{-9}
16	0.21	12,300	10^{-9}
24	0.25	7,200,000	10^{-8}
36	0.43	310,000,000	10^{-9}
48	0.68	660,000,000	10^{-8}

The titrable acidity, number of bacteria and bacteriophage titer was followed with four other vats of skim milk used in the manufacture of cottage cheese and in which acid production was slow due to the presence of bacteriophage. The same general results as those given in table 3 were obtained. In all cases acid production was slow for about 24 hrs.; later, a more or less normal acid production occurred. The number of bacteria generally showed a slight increase which was followed by a very large decrease and then a large increase. The bacteriophage titer commonly showed an increase during the first 7 to 12 hrs. after setting; after this period the titer remained practically constant.

DISCUSSION

In all of the trials in which slow acid production during the manufacture of cheddar cheese was due to bacteriophage, acid development appeared to be nearly normal until the time of draining the whey. However, only slight increases normally are observed in the titrable acidity during this period and titration values frequently are confusing since those obtained on milk are not comparable to those obtained on whey. A rapid increase in the whey acidity takes place after the whey is drained with normal working vats. When such an increase in whey acidity fails to take place, it is easily recognized. For experimental purposes it might be desirable to follow the pH rather than the titrable acidity since removal of the casein from solution would not alter its value. The cultures selected for all of the trials were checked for their ability to produce acid at the cooking temperature employed (102° F.); therefore, temperature was not an influencing factor. When acid production is somewhat slow during the manufacture of cheddar cheese, many cheesemakers prefer to cheddar for a longer period of time so that each vat will be milled at about the same acidity. When bacteriophage was the cause of slow acid production, the curd commonly could be cheddared for very long periods of time (4 to 5 hrs.) without an appreciable increase in acidity.

The action of a bacteria-free whey filtrate (obtained from a vat of cheddar cheese in which acid production was slow) containing bacteriophage on pure cultures of *S. lactis* (obtained from the cheese culture employed in the same vat) is shown by data presented in table 2. Since the data show that 20 per cent (3 of 15 cultures) of the *S. lactis* cultures were not affected by bacteriophage, it would be presumed that these bacteriophage-resistant cultures would produce an appreciable amount of lactic acid. However, this was not the case in actual cheesemaking operations. Considerable variation was shown among the various sensitive strains of *S. lactis* in their ability to produce secondary growth. In the manufacture of cheddar cheese the time of manufacture could not be increased sufficiently to observe the effects of secondary growth.

The literature on bacteriophage as a cause of slow acid production in cheesemaking is largely associated with the manufacture of cheddar cheese. In this country, where a considerable amount of cottage cheese is manufactured, bacteriophage difficulties can be expected. Since cottage cheese is prepared from acid-coagulated skim milk, lactic acid production by the cheese culture is perhaps more important than in any other type of cheese. The data presented on the effect of bacteriophage on acid production by a cheese culture during the manufacture of cottage cheese would be expected to be very similar to data obtained when the bulk culture becomes contaminated with bacteriophage and fails to coagulate. As in the manufacture of cheddar cheese, acid production appears quite normal and there is evidence of growth of the culture organisms soon after setting. Later, however, acid fails to develop, the bacteria count decreases and the bacteriophage titer increases. Still later, the secondary growth of the culture organisms is manifest, and from then on acid production and the increase in number of bacteria proceeds in a more or less normal manner. If the skim milk intended for cottage cheese is set in the usual way and acid fails to develop as the result of bacteriophage, the skim milk will coagulate if left in the vat long enough. In many of the trials in which slow-acid production was encountered during the manufacture of cottage cheese, the skim milk was allowed to stand in the vat for periods of 40 to 50 hrs. before the milk had coagulated and the acid had reached a whey acidity of 0.50 per cent. Although a long setting period was required to develop sufficient acid, the cottage cheese finally obtained usually was of good quality and in most instances could not be distinguished from cottage cheese in which acid development had proceeded normally (14 to 16 hrs.). The initial bacteriological quality of the skim milk is an important factor in this respect.

SUMMARY

Slow acid production, due to the presence of bacteriophage, in the manufacture of cheddar cheese usually was apparent at the time of draining the whey or shortly thereafter.

The presence of bacteriophage resulted in almost complete cessation of acid production in the manufacture of cheddar cheese when either single-strain or multiple-strain cheese cultures were employed.

Cheddaring for a long period of time (4 or 5 hrs.) did not result in an appreciable increase in acidity when acid production was slow because of bacteriophage action.

The presence of bacteriophage in bacteria-free filtrates of whey, culture, etc., could be demonstrated by adding a small amount of the filtrate to pure cultures of *S. lactis* isolated from the culture used in the manufacturing process and also by the production of bacteriophage plaques on a solid medium.

When a vat of skim milk intended for cottage cheese was contaminated slightly with bacteriophage active against the culture employed, acid formation was very slow for about 24 hrs. after setting. During this same period the bacteria count showed a slight increase, then a large decrease, and finally a large increase. The bacteriophage titer increased for 7 to 12 hrs. following setting and then remained practically constant. When secondary growth occurred (usually after 24 hrs.), acid production proceeded normally.

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THE MANUFACTURE OF STERILIZED CARAMEL MILK

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The evaporated milk industry during World War II was interested in methods of preparing canned milk drinks of pleasing flavor and long storage life for the armed forces. The beverages were to be used hot or cold and without dilution with water and they were to act as a quick source of energy on invasion beachheads when appetites were sluggish. The caramel-flavored milk described in this paper was the best of a wide variety of milks which were studied in these laboratories in cooperation with an evaporated milk manufacturer who produced several commercial size batches.

EXPERIMENTAL

Test mixtures were prepared by adding the flavor base or concentrate to the milk, then homogenizing, canning and sterilizing the products. Sterilization was accomplished in a pilot evaporated milk sterilizer at 240° F. for 15 minutes, with the reel revolving at 4 r.p.m. Pilot plant tests of promising formulas were conducted in an evaporated milk plant by the cooperating manufacturer.

An important phase of the problem was the development of a suitable caramel base for flavoring the milk. The bases were prepared by following conventional caramel making procedures. The caramel mix was cooked in a steam-jacketed candy kettle equipped with a double action stirrer.

Viscosity determinations were made at 86° F., the storage temperature, with a McMichael viscosimeter equipped with standardized wires.

RESULTS

Preliminary tests indicated that milks flavored with fruits, maple and sweetened vanilla were less attractive or possessed poorer storage qualities than did chocolate or caramel milks. Work was centered on caramel flavor because caramel is present to some extent in all sterilized milk and since there appeared to be a possibility of developing a new and pleasing caramel-flavored milk drink.

It was found that a pronounced caramel flavor could not be developed during the sterilization of a milk sweetened with dextrose or sucrose. Such milks had a cooked flavor similar to that of evaporated milk. More heat and higher concentrations of proteins and sugars than in normal processing were necessary to produce a caramel flavor. When milk protein was partially hydrolyzed by continuously heating it in an acid medium (below pH 6.3) a

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cooked meat flavor was produced, but when a sugar was present in the mixture a caramel flavor resulted. The best caramel flavor was developed when a milk and sugar mixture was concentrated by boiling in an open kettle by methods used by caramel manufacturers.

Experiments were directed toward the preparation of a caramel base which would be suitable for use in the manufacture of caramel milk. The flavoring base finally produced was similar to a caramel topping. It was more fluid than caramel candy. The formula for this base is given in table 1. Combinations of various dairy products were used to fill the fat, milk-solids-not-fat and sugar needs of the formula. A sweeter mix was produced when the corn sirup was decreased about 25 per cent and the sugar increased as much as 80 per cent of the values given in table 1. If the fat content of the base was decreased and the milk-solids-not-fat content increased until the two values were equal, the base was inferior to the high-fat base in flavor but otherwise it was satisfactory. Caramel bases to which less water was added after cooking were made but these were too heavy to flow easily. When the caramel was not diluted with water after cooking, it contained about 89 per cent total solids.

TABLE 1

Formula for caramel flavor base

Ingredient	Wt. of ingredient	Weights of components		
		Fat	SNF	T.S
	lbs.	*lbs.*	*lbs.*	*lbs.*
Cream (30% fat)	33.3	10	2.1	12.1
Condensed skim milk (30% solids)	15.0		4.5	4.5
Corn sirup	45.0			36.0
Sucrose	14.3			14.3
Salt	0.7			0.7
Total	108.3	10	6.6	67.6

Heat the complete mixture with stirring to 160° F., and homogenize it at 2500 lbs. pressure. Cook in a candy kettle with double action stirrer to 238° F., cool slightly and add 8¼ lbs. of water and vanilla flavoring to suit the taste. Stir well and pour into a suitable receiver. Approximately 84 pounds of base containing 20% water will be recovered.

Caramel milk was manufactured according to the formula of table 2. The simplest method of preparing trial batches was to mix 14 pounds of base with 42½ pounds of water and 43½ pounds of 26 per cent evaporated milk that had not been sterilized. The body and flavor of the product were better when the proper concentrations of plain and concentrated milks were used in place of water and 26 per cent solids milk. A satisfactory procedure was to supplement a plain milk with one concentrated to 15 or 20 per cent solids content. Under such conditions approximately one-third of the milk would be partially concentrated milk from a regular evaporated milk operation while the other two-thirds would be untreated whole milk. The milk solids requirement of the formula of table 2 was also satisfield when the 86 pounds of milk in the formula were added as milk containing 4.07 per cent fat and 9.18 per cent solids-not-fat.

The forewarming treatment given the milk before it was mixed with the caramel base affected the heat stability of the finished product as shown in

TABLE 2

Formula for caramel milk

Ingredient	Weight of ingredient	Weights of components				
		Fat	M.S.N.F.	Sucrose	Corn sirup solids	Total solids
	lbs.	*lbs*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Caramel base (table 1)	14	1.7	1.1	2.3	6.0	11.1
Milk	86	3.5	7.9			11.4
Total	100	5.2	9.0	2.3	6.0	22.5

TABLE 3

The effect of the heat treatment of milk upon the heat stability of caramel milk. Milk of 4.0 per cent fat and 9.2 per cent solids-not-fat was used. Homogenization was at 2500 pounds per sq. in. pressure

Forewarming treatment		Condensed to T.S.	Preparation of mixture				Heat stability at 240° F.
			Composition			Mixing and homogenization temperature	
Temp.	Time		Milk	Water	Caramel (table 1)		
°F.	*min.*	%	*lbs*	*lbs.*	*lbs.*	*°F*	*min.*
203	10	26	43½	42½	14	140	58
None		Not condensed	86	0	14	140	51
203	10	Not condensed	86	0	14	180	90

TABLE 4

The effect of storage at 86° F. upon the viscosity of commercial samples of evaporated milk and of caramel flavored milk

Milk sample*	Fat	T.S.	Heat stability at 240° F.†	Viscosity at 86° F.					
				1 day	10 days	30 days	80 days	200 days	365 days
	%	%	*min.*	*cp.*	*cp.*	*cp.*	*cp.*	*cp.*	*cp.*
Evap.	7.96	26.05	12	51	43	30	27	22	25
Caramel 1	4.92	23.39	30	12	11	10	10	11	13
Caramel 2	4.93	22.93	10	51	57	55	56	55	57

* Each of the three batches were sterilized at 240° F. for 15 minutes. Samples were received and placed in storage at 86° F., 15 to 20 days after date of manufacture.

† The heat stability figures are estimated from the appearance and viscosity of the milk examined after sterilization. Facilities were not available for making accurate heat stability tests on these milks.

table 3. The milks used in this experiment were more heat stable than the commercial milks of Table 4.

A comparison of the viscosity changes of normal evaporated milk and of caramel milk during storage at 86° F. is given in table 4. Caramel milk #1 was compounded from 44 parts of 26 per cent evaporated milk (not sterilized), 14 parts of caramel base (table 1) and 42 parts of water. Caramel milk #2 was made from a batch which contained 96 pounds of a heavy caramel base (T.S. 89%), 224 pounds of concentrated milk (T.S. 20%) and 480 pounds of raw fresh milk (T.S. 12%).

DISCUSSION

The caramel flavor of the finished milk was strong enough to overcome the cooked flavor which developed during sterilization. Caramel milk was found to possess an attractive flavor when it was used either hot or cold. Bases made with fats other than milk fat did not give the finished milk the delicate milk flavor produced by milk-fat bases. The fat content of caramel milks was varied from 2 per cent to 5 per cent but the flavor was superior in the high-fat milks. The vanilla added to the caramel base helped to point up the flavor of the milk.

The viscosity of caramel milk ranged from 8 to 65 centipoises at 86° F., about 25 centipoises being the optimum viscosity for a free flowing beverage. However, the more viscous milks were protected from fat separation and caramel precipitation much better than the thin ones. Caramel milk 1, table 4, was low in viscosity. After storage for a year at 86° F. it showed definite fat separation and there was a quarter-inch layer of precipitate in the bottom of the can. Caramel milk 2 was more viscous and had neither fat separation at the top nor sediment at the bottom of the can after storage under similar conditions. A viscosity in excess of 50 centipoises at 86° F. was required to prevent separation in milks which were held in storage for long periods.

The viscosity of the finished milk was controlled by adjusting the heat stability of the milk before sterilization as is done in the manufacture of evaporated milk (1). Forewarming the raw milk to high temperatures produced high heat stability (coagulation at 240° F only after 30 minutes or longer) and a low viscosity in the sterilized milk, while low forewarming temperatures had the opposite effects.

The persistence of viscosity in caramel milks during storage was of particular interest. The viscosities of evaporated milks, illustrated by the sample shown in table 4, decreased to about one-half their initial values during the first few months' storage at temperatures of about 86° F. or higher. The viscosities of four samples of commercially produced caramel milk which were placed in storage at 86° F. within 3 weeks of the date of manufacture did not decrease further during one year. Data on two of these samples appear in table 4. The viscosity of four samples of caramel milk prepared in the Research Laboratories and held at 86° F. showed an average decrease to 84 per cent of their initial viscosity from the date of manufacture to one year thereafter.

The factors responsible for preserving the viscosity in caramel milk were not determined nor are the causes of the change in evaporated milk understood.

SUMMARY

A process was developed for the preparation of a caramel-flavored sterilized milk for use as a beverage by the armed forces. The milk was made by mixing a caramel base with plain and concentrated milk to produce a product containing 5 per cent milk fat and 22 per cent total solids. The mixture was canned and processed by the same procedure as is used for evaporated milk. Some caramel milks were more stable than samples of evaporated milk in flavor, viscosity and body during storage for one year at 86° F.

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DIGESTIBILITY OF KOBE LESPEDEZA HAY

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Louisiana and other southern states have made greater usage of lespedeza for pastures and hay in recent years. Common or Japanese lespedeza (*Lespedeza striata*) is one of the most extensively grown legumes. Kobe lespedeza, a variety of common lespedeza, is gaining in popularity, especially because of its higher yield of hay.

This paper reports the digestibility of Kobe lespedeza hay cut at three stages of maturity.

MATERIAL AND PROCEDURE

Kobe lespedeza hay, approximately 90 per cent pure, was obtained in 1945 from a farm near Baskin in Franklin Parish. The soil on which the hay was grown is of medium fertility. The field was fertilized with 400 pounds of 4–12–4 fertilizer before oat-planting and the Kobe lespedeza was seeded during late winter in the oats. The first cutting of hay was made slightly earlier than the full-bloom stage, the second cutting was made during early-seed stage, and the third in the full-seed stage. This last cutting produced very poor quality hay. The hay was chopped to about one-inch lengths for feeding. Four dairy steers weighing from 400 to 500 pounds were used as experimental animals in the digestion trials.

The procedure used in management of the animals and in obtaining samples of hay and excreta has been reported in a recent paper (3). In general, it included the use of a 10-day preliminary period and a 10-day collection period. The feces were collected in a canvas collection bag. A 1/50 aliquot was taken from each day's fecal output for each steer and stored in a refrigerator at 0° C. until completion of the collection period. Nitrogen determinations were made on aliquots of the mixed fresh excreta, while dried excretum samples were used for the other analyses. The A.O.A.C. methods (2) were employed for the usual chemical analyses of hay and excreta, while lignin and cellulose were determined by the method of Crampton and Maynard (1).

Nine to eleven pounds of hay was fed daily, with very little feed being refused. The steers maintained their weights throughout the experiment.

RESULTS AND DISCUSSION

The composition, coefficients of digestibility, and digestible nutrients of Kobe lespedeza hay at the bloom, early-seed, and full-seed stages are presented in table 1.

The bloom-stage hay was the most desirable because of its higher percentages of crude protein, nitrogen-free extract, and ash. This hay was also

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higher in digestible protein and total digestible nutrients. The percentages of crude fiber and lignin increased with advance in stage of maturity of the hay, while the nitrogen-free extract decreased. The more matured hay was a relatively poor source of nutrients, particularly of protein.

A comparison of values found for Kobe lespedeza hay in this study and common lespedeza hay reported previously (3) shows that at similar stages of maturity the composition and digestible nutrients are almost identical. The percentages of crude protein and total digestible nutrients for the Kobe lespedeza in the early-cut stage (bloom) were 10.48 and 50.06, as compared to 10.52 and 50.06 for common lespedeza hay. In the early-seed or intermediate stage the values for protein and total digestible nutrients for Kobe lespedeza were 8.21 and 49.22, while those of the common lespedeza were 8.55 and 48.28. Kobe lespedeza hay cut in the very late stage (full-seed) contained 6.15 per cent protein and 40.70 total digestible nutrients. The coefficient of digestibility and percentage of digestible crude protein for this stage of maturity were surprisingly low, being 3.60 and 0.22 per cent, respectively, as compared to 47.55 and 4.98 per cent when the hay was cut in the bloom stage.

TABLE 1

The composition, digestion coefficients, and digestible nutrients of Kobe lespedeza hay at three stages of maturity (dry basis)

Stage of hay	Dry matter	Crude protein	Crude fiber	N-free extract	Crude fat	Lignin	Cellulose	Ash
	%	%	%	%	%	%	%	%
Bloom	94.30	10.48	42.09	41.60	1.60	26.04	37.47	4.23
Early-seed	92.07	8.21	45.88	40.57	1.41	27.10	41.34	3.93
Late seed	93.51	6.15	50.35	38.76	1.57	30.20	36.25	3.16
				Digestion coefficients				
Bloom		47.55	46.78	65.37	31.99	18.00	49.54	
Early-seed		32.29	45.11	63.26	7.08	20.00	57.70	
Late seed		3.60	36.74	53.96	30.99	13.00	36.16	
				Digestible nutrients				
						T.D.N.		
Bloom		4.98	18.00	26.11	0.43	50.06		
Early-seed		2.65	20.69	25.66	0.10	49.22		
Late-seed		0.22	18.50	20.91	0.48	40.70		

The values for the late-cut mature hay are unreasonably low and difficult to explain. It was expected that the values for the coefficient of digestibility and digestible protein would approximate 34.53 and 2.14 per cent, the values for low quality, late-cut native grass hay (4). This grass hay showed a protein content of 6.21 per cent on the dry basis and a total digestible nutrient value of 40.26, which are practically the same as the values for the late-cut Kobe lespedeza hay. The relatively low crude protein, however, definitely classifies this late-cut lespedeza hay as of inferior quality.

In a previous paper (3) a comparison was made of the digestibility values reported for common lespedeza and Korean lespedeza hay (5) at similar stages of maturity. The figures reported for percentage of crude protein in the Korean lespedeza are slightly higher than those for either the Kobe or common lespedeza at the same stage of maturity, *i.e.*, intermediate stage 11.84, 10.52, and 10.48, respectively. One explanation for this might be the practice of the Louisiana farmer, because of a long growing season, to postpone haying until fall when more favorable hay-curing weather exists. The rainy weather conditions make early cutting of hay more hazardous.

The total digestible nutrients on the dry basis for Kobe lespedeza, common lespedeza (3), and Korean lespedeza (5) at the same stages of maturity are very similar. In the bloom stage the values are 50.06, 50.06, and 48.51, respectively, and in the late stage of maturity the values are 48.22, 48.28, and 43.66, respectively.

The lignin and cellulose values for Kobe lespedeza hay are higher than those reported for Korean (5) and common lespedeza hay (3). The possible relationship between lignin content and feed utilization is again demonstrated in this study, for with an increase in lignin value there was a decrease in total digestible nutrients.

SUMMARY

Kobe lespedeza hay cut at three stages of maturity—bloom, early-seed, and late-seed—was used in three digestion trials with four dairy steers. Percentages of crude protein on the dry basis were 10.48, 8.21, and 6.15; percentages of digestible protein were 4.98, 2.65, and 0.22; and the total digestible nutrients were 50.06, 49.22, and 40.70 for the bloom, early-seed, and late-seed stages, respectively. The early-cut hay proved more valuable because of its higher nutritive value. These values are very similar to those of common lespedeza hay at the same stages of maturity.

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STUDIES ON MILK FEVER IN DAIRY COWS[1]

I. THE POSSIBLE ROLE OF VITAMIN D IN MILK FEVER

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Since the early 1920's milk fever, or parturient paresis, in dairy cows has been the subject of considerable research and theoretical discussion. Numerous theories regarding the etiology of milk fever have been advanced, namely anaphylaxis, circulatory disfunction, the presence of air in the blood, cerebral anemia, anhydremia (16) infection (29), auto-intoxication (18, 25), defective oxidation in the tissues (7, 8), excess oxytocic principle in the blood after parturition (4), hypoglycemia (2, 6, 24, 32, 33, 34, 38, 39), and hypocalcemia associated with disturbed mineral metabolism (3, 5, 8, 9, 10, 12, 13, 14, 15, 21, 23, 31, 36, 37, 40). A review of theories up to 1926 is presented by Little and Wright (22).

Little experimental support is offered for any of these theories, except for the hypoglycemic and hypocalcemic theories. Several workers have published results which would tend to disprove the hypoglycemic theory (7, 17, 26, 27). Therefore, it appears that the answer to the question of the basic cause of milk fever must lie in the realm of disturbed mineral metabolism.

This view is further substantiated by the changes occurring in the blood at parturition and during the milk fever attack. A marked decrease in both total calcium (1, 3, 8, 9, 10, 11, 12, 14, 21, 23, 35, 37, 40) and ionized calcium (12, 23, 31, 37) has been reported. Phosphorus is greatly decreased (3, 9, 10, 11, 12, 23, 28, 35, 37, 40) in milk fever and in contrast to calcium and phosphorus, magnesium is said to increase (1, 3, 12, 28, 30, 31, 37).

Furthermore, milk fever treatment, whether it be udder inflation or intravenous injection of calcium salts, is primarily concerned with elevating the blood calcium to the normal level. It would seem, therefore, that any prophylactic method, to be effective, should prevent the fall of blood calcium below the critical level.

That vitamin D may be a factor in milk fever is suggested by the observation that the highest incidence is reported to occur in the winter and early spring months (19, 26) when solar irradiation is the least.

The role of vitamin D in calcium and phosphorus metabolism has been recognized for many years. Since vitamin D is known to have calcemic properties and because it is now available in cheap, abundant quantities it

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seemed reasonable that the feeding of large amounts of vitamin D prior to parturition might prove beneficial in the control of milk fever. Greig (14) and Sjollema (36) have suggested the feeding of vitamin D as a possible preventive measure in milk fever but give no experimental evidence of its value. An increase in blood calcium in cows receiving amounts of vitamin D above their actual needs is reported by Hess *et al.* (20). The authors report no pathological effects in the tissues of cows fed 1,500,000 units of vitamin D per day.

Herein reported are the results of experiments designed to determine whether or not the feeding of massive doses of vitamin D prior to parturition will lower the incidence of milk fever.

The results of the effect of vitamin D feeding on the blood changes at parturition and in milk fever will be presented in another paper.

EXPERIMENTAL

In a preliminary experiment, six cows were fed 1 million units of vitamin D daily in the form of type (7 F) irradiated dry yeast[3] for four weeks before the expected date of parturition and for one week following parturition. Although two of these cows had previous histories of milk fever, none of them developed milk fever. Two of the five control animals developed milk fever. Both of these cows had previous histories of milk fever.

As a result of this preliminary trial a more extensive experiment was planned in which the main Experiment Station, the Station Pasture Farm, and the Ohio State University herds were used. During the first 2 years (1941–43) all cows exclusive of first-calf heifers were included. Later (1943–45) only the two Experiment Station herds were used and only Jersey cows that had had at least two previous parturitions were included.

Beginning September 15, 1941, the cows included in the experiment were divided into two equal groups as nearly comparable as possible, based on breed, age, expected freshening date, and previous milk fever histories. One group was fed vitamin D and the other, which served as a control, was not fed vitamin D. At each successive freshening, the cows were assigned to the opposite group. Thus each cow served as her own control for several freshenings, if she remained in the herd.

Hay, silage, grain, and pasture were fed throughout the year according to supplies available and the practice commonly employed in the various herds. All cows in each herd were fed and managed alike during the dry period and after parturition except for the yeast supplement.

In this experiment vitamin D was supplied in the form of type (9F) irradiated dry yeast,[4] 110 grams being required to supply 1 million U.S.P. units.

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[3] Supplied by Standard Brands Incorporated, New York City.

[4] Supplied by Standard Brands Incorporated, New York City.

TABLE 1

The effect of irradiated-yeast feeding on the incidence of milk fever in different breeds of dairy cows

	Total parturitions	Yeast-fed	Control
Ayrshire			
Fresh, normally	18	8	10
Milk fever	3	2	1
Total parturitions	21	10	11
Per cent incidence	14.3	20.0	9.1
Guernsey			
Fresh, normally	13	6	7
Milk fever	2	2	0
Total parturitions	15	8	7
Per cent incidence	6.7	25.0	0.0
Holstein			
Fresh, normally	35	12	23
Milk fever	2	1	1
Total parturitions	37	13	24
Per cent incidence	5.4	7.8	4.2
Jersey			
Fresh, normally	70	36	34
Milk fever	35	17	18
Total parturitions	105	53	52
Per cent incidence	33.3	32.1	34.6
All Breeds			
Fresh, normally	136	62	74
Milk fever	42	22	20
Total parturitions	178	84	94
Per cent incidence	23.6	26.2	21.3

Beginning 4 weeks before the expected date of parturition and continuing for 1 week following parturition, 1 million U.S.P. units of vitamin D were fed daily by sprinkling one half of the allowance on the grain at each feeding. In a very few cases, the cows were slow at first in eating their grain which contained the irradiated yeast but most of them ate it readily from the start.

A careful record was kept of the occurrence of milk fever, based on the diagnosis of a veterinarian and on blood analyses. The results of feeding the previously indicated amount of vitamin D in the form of irradiated dry yeast on milk fever incidence are indicated in tables 1 and 2.

The seasonal incidence of milk fever based on the 105 parturitions in

TABLE 2

The effect of irradiated yeast feeding on the incidence of milk fever in Jersey cows with previous milk fever histories

	Total parturitions	Yeast-fed	Control
Fresh, normally	19	13	6
Milk fever	21	10	11
Total parturitions	40	23	17
Per cent incidence	52.5	43.5	64.7

mature Jersey cows included in this study is presented in table 3. It will be seen that essentially the same per cent incidence of milk fever occurred in the period from May through September as in the period October through April.

This observation is at variance with that of Metzger and Morrison (26) and Henderson (19) who report a higher per cent incidence of milk fever in the winter than in the summer. No explanation is evident for this difference; however, it seems reasonable that solar irradiation may not have as much effect on lowering milk fever incidence as is commonly believed since the highest vitamin D potency of both blood and milk under summer pasture conditions is considerably less than when 1 million units of vitamin D is administered daily in the form of irradiated yeast.

TABLE 3

Seasonal incidence of milk fever in mature Jersey cows

	Normal	Milk fever	Total	Per cent milk fever
January	5	1	6	16.7
February	16	10	26	38.4
March	20	9	29	31.0
April	7	3	10	30.0
May	3	1	4	25.0
June	3	1	4	25.0
July	1	1	2	50.0
August	3	1	4	25.0
September	3	3	6	50.0
October	2	2	4	50.0
November	3	2	5	40.0
December	4	1	5	20.0
Total	70	35	105	33.3
May–September	13	7	20	35.0
October–April	57	28	85	33.0

DISCUSSION

It will be noted that the incidence of milk fever is much higher in the Jersey breed than in the other breeds. The average per cent incidence of milk fever in breeds other than Jerseys was 9.6 compared to 33.3 in the Jerseys. This is in agreement with data published by Metzger and Morrison 1936 (26) and Henderson 1938 (19).

All the data involving Ayrshires and Guernseys, plus 16 of the 35 Holstein cows, were obtained from the Ohio State University herd. All except five Jersey parturitions occurred in the Experiment Station herd. The milk fever cases in the Holstein, Guernsey, and Ayrshire breeds occurred in the Ohio State University herd. Milk fever has not been observed in the Experiment Station Holstein herd in recent years.

An examination of the data from all four breeds shows that the feeding of 1 million units of vitamin D in the form of irradiated yeast did not prevent milk fever. The difference in per cent incidence between yeast-fed and control cows of the Ayrshire and Guernsey breeds is not considered to be significant due to the small numbers involved.

In considering the results when limited to Jersey cows with previous milk fever histories, it appears that some beneficial effect can be attributed to the treatment. Of 40 such parturitions, only 43.5 per cent of the cows in the yeast-fed group had milk fever as compared to 64.7 per cent in the control group.

An examination of the data regarding the effect of irradiated yeast feeding on the blood calcium and phosphorus, to be presented in another paper, indicates no significant difference between the yeast-fed and the control group. This substantiates the results of the incidence study.

The results of this experiment are not interpreted to mean that vitamin D has no place in the control of milk fever. It is entirely possible that if larger amounts of vitamin D had been consumed, the effect would have been greater. Preliminary studies with larger amounts of vitamin D indicate that it takes more than 1 million units daily to raise the calcium and phosphorus levels of the blood materially. It is possible that with higher levels of vitamin D feeding milk fever incidence could be reduced. Such studies are now in progress.

SUMMARY AND CONCLUSIONS

1. The feeding of 1 million units of vitamin D daily in the form of irradiated yeast for 4 weeks prior to and one week following parturition did not reduce the incidence of milk fever in an experiment involving 178 parturitions in four breeds of dairy cows.
2. The highest incidence of milk fever was observed in Jersey cows.
3. Milk fever occurred only in cows with at least two previous parturitions. The percent incidence in mature Jersey cows was found to be equally as high in summer as in winter.
4. The irradiated yeast treatment reduced the incidence of milk fever somewhat in Jersey cows which had previous histories of milk fever.

Experiments to investigate the effect of feeding larger amounts of vitamin D are in progress.

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ADDITIONAL OBSERVATIONS ON THE STABILITY OF ASCORBIC ACID AND SODIUM L-ASCORBATE IN EVAPORATED MILK*

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Previous studies have been published dealing with the ascorbic acid content of commercially manufactured evaporated milk (1) and with the stability of the vitamin in fortified and unfortified evaporated milk, sealed normally and after removal of the air from the can (2, 3). Storage results were given up to 12 months at room temperature in the latter studies. Extra samples of a number of the batches made were available and have since been analyzed after 24 and 28 months of holding, thus extending the data significantly.

One study, utilizing sodium l-ascorbate in place of ascorbic acid, has now been carried to 12 months of storage and the data can be compared with the previously obtained results. The sodium salt was used in an effort to avoid the destabilizing effect of the ascorbic acid on the milk protein when fortification at high levels (100 mg./l. and over, fluid basis) was attempted. This effect manifests itself in a flaking of the protein during the sterilization of the milk and requires additional quantities of stabilizing salts (di-sodium phosphate or sodium citrate) to overcome it.

Methods and procedures employed in the studies have been described in the previous publications and will not be given here.

In the accompanying figure, the average reduced ascorbic acid levels of the variously treated batches of evaporated milk are plotted against storage time in months at room temperature. The curves depicting the loss of the vitamin on holding are remarkably uniform, and the rates of loss nominal. Vacuum sealed milk containing 106.5 mg. l. (fluid basis) of ascorbic acid after sterilizing still retained 71.2 mg., 24 months later Similar milk with 58.6 mg. to begin with had 32.6 mg., 28 months later. These data further emphasize the suitability of evaporated milk as a medium for fortification with and storage of vitamin C.

The loss of ascorbic acid in evaporated milk in milligrams per month is higher for milk rich in ascorbic acid and lower in milk poor in the vitamin, as can be noted from the curves. After the first two to four months, during which time the free oxygen in the container apparently reacts with the ascorbic acid and particularly with the heat-generated reducing substances (3), the rate of loss of the vitamin becomes much slower. The loss per interval

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of time, however, seems to be proportional to the quantity of ascorbic acid present. This is made clearer in table 1 where the losses suffered by a number of variously treated batches of evaporated milk during the last twelve months of storage are shown. The percentage loss on all these batches ranges from 11.4 to 16.6 which is considered quite uniform and rather significant in view of the diversity of treatment. It is apparent, of course, that this rate of loss, approximately 14 per cent, applies only to the storage period subsequent to the first two to four months of more rapid loss as is evident in the figure. Even in the first period, however, the loss of ascorbic acid appears to be more closely related to the amount present than to the available oxygen since the normally sealed, unfortified, evaporated milk exhibited only a small loss in milligrams compared to the normally sealed fortified milk and the vacuum-sealed fortified milk showed a greater loss than the normally sealed unfortified milk. Thus, it appears that the oxidation of reduced ascorbic acid in evaporated milk is not a quantitative reaction with free oxygen as has been found true with sealed samples of fluid milk by Sharp *et al.* (4) and Noll and Supplee (5). The results presented in the figure and in table 1 show very conclusively that vacuum sealing has no effect on the rate of loss after the first two to four months period.

TABLE 1

The loss of ascorbic acid in evaporated milk during the last twelve months of storage at room temperature

Treatment of milk	Total time stored (months)	Ascorbic acid (mg./l.—fluid basis) last 12 months of period			
		Beginning	End	Loss	Per cent loss
Not fortified					
1. Normal seal	18	7.0	6.2	0.8	11.4
2. Vacuum seal	18	10.1	8.9	1.2	12.0
50 mg./l. fortification					
3. Normal seal	28	32.5	27.5	5.0	15.4
4. Normal seal (sodium salt)	12	36.5	31.5*	5.0	13.7
5. Vacuum seal	28	42.0	35.0	7.0	16.6
100 mg./l. fortification					
6. Normal seal (underfilled)	24	40.5	33.8	6.7	16.5
7. Normal seal	24	67.1	57.0	10.1	15.0
8. Normal seal (sodium salt)	12	75.4	65.0*	10.4	13.8
9. Vacuum seal	24	81.3	71.0	10.3	12.7

* These data were obtained by extrapolating the values for the last six months.

Sodium *l*-ascorbate was found to be more satisfactory as a fortifying substance for addition to evaporated milk than the acid form of the vitamin because it does not destabilize the milk proteins toward heat and create sterilizing difficulties when levels of 100 mg./l. (fluid basis) and above are used. This is indicated by the data in table 2. A batch of milk was con-

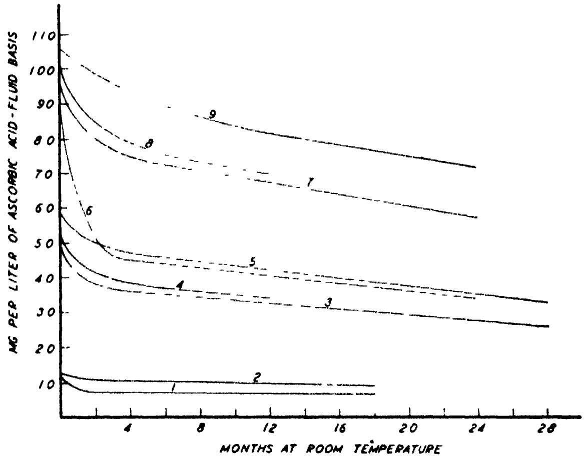

FIG. 1. Effect of storage at room temperature on the ascorbic acid content of variously treated evaporated milk. 1. Not fortified—normal seal. 2. Not fortified—vacuum seal. 3. Fortified (50 mg./l. ascorbic acid)—normal seal. 4. Fortified (50 mg./l. sodium *l*-ascorbate)—normal seal. 5. Fortified (50 mg./l. ascorbic acid)—vacuum seal. 6. Fortified (100 mg./l. ascorbic acid)—underfilled—normal seal. 7. Fortified (100 mg./l. ascorbic acid)—normal seal. 8. Fortified (100 mg./l. sodium *l*-ascorbate)—normal seal. 9. Fortified (100 mg./l. ascorbic acid)—vacuum seal.

centrated to a point representing the threshold of coagulation at 240° F. for 15 minutes. It was then partitioned and fortified with 50, 100, and 150 mg./l. of ascorbic acid respectively, using the acid form of the vitamin and the sodium salt. The cans were sealed and sterilized normally.

The data reveal that no detectable change in the visible amount of coagulation occurred when sodium *l*-ascorbate was used in evaporated milk as the fortifying agent, whereas the ascorbic acid aggravated the coagulation very

TABLE 2

Effect on sterilizing characteristics of evaporated milk when fortification is accomplished with ascorbic acid and with sodium l-ascorbate

Treatment of milk	Ascorbic acid (fluid basis)		Degree of coagulation	pH
	Before sterilization	After sterilization		
	mg./l.	*mg./l.*		
Control*	11.9	9.8	±	6.19
50 mg./l. ascorbic acid	58.8	53.2	+	6.19
100 mg./l. ascorbic acid	109.0	99.2	++	6.18
150 mg./l. ascorbic acid	156.9	144.5	+++	6.18
50 mg./l. sodium *l*-ascorbate	58.5†	53.6	±	6.19
100 mg./l. sodium *l*-ascorbate	109.3	102.0	±	6.19
150 mg./l. sodium *l*-ascorbate	157.9	143.0	±	6.20

* Milk was concentrated to 19.92 per cent solids not fat.

† Titration indicated the sodium salt to contain 88.4 per cent ascorbic acid, the amount used therefore was 1.131 times the amount of the acid.

definitely as the concentration of the vitamin was increased. Variations in pH of the milk as a result of the vitamin additions were hardly in excess of the sensitivity of the instrument used (Beckman pH meter). Table 2 shows no significant differences in the effect of the sterilizing treatment on the vitamin, whether ascorbic acid or its sodium salt was employed for fortification and the curves in the figure indicate that the storage losses follow an identical trend with the previously studied batches of milk.

FINAL CONCLUSIONS

Evaporated milk is a highly suitable medium for fortification with ascorbic acid (vitamin C) inasmuch as the losses during storage are small and predictable.

The disappearance of ascorbic acid in evaporated milk after the first two to four months of storage is uniform and proportional to the amounts present.

Oxidation of ascorbic acid in evaporated milk is not a quantitative reaction with the free oxygen present as is apparently the case with sealed samples of fluid milk.

The sodium salt of ascorbic acid is a more satisfactory form of the vitamin to use for fortifying evaporated milk inasmuch as it does not effect the stability of the porteins toward sterilization and in all other respects behaves identically with the acid.

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THE USE OF UREA IN RATIONS FOR DAIRY COWS UNDER HAWAIIAN CONDITIONS*

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Research conducted at experiment stations in the United States (1, 7, 18) and in other countries (3, 17) has demonstrated that urea has considerable value as a substitute for high-protein concentrates in rations for lactating cows. The results have been so satisfactory, in fact, that the Association of American Feed Control Officials have approved its inclusion in feeds for ruminants, and it has been widely used.

Early work conducted at the Hawaii Agricultural Experiment Station (21) showed that urea could be utilized by dairy heifers for growth. Balance studies conducted by Harris, Work, and Henke (6) with four steers indicated that the nitrogen from urea was as digestible as that from soybean oil meal but that its biological value was lower. Trials were also initiated to determine its usefulness in rations for dairy cows under Hawaiian conditions. Because of the shortage of ordinary high-protein concentrates, this project assumed considerable importance during the war. In two double-reversal trials using a total of twenty cows, significantly lower milk production was obtained when urea replaced part of the soybean oil meal in the control ration (11). The results of four more recent trials with lactating cows are reported in this paper. The first two were designed to give more conclusive information on the value of this synthetic product. One additional trial measured the value of urea fed at two different levels. Another determined the effect of large amounts of molasses upon the utilization of urea.

GENERAL PLAN OF TRIALS

The trials reported herein were of the double change-over type as designed by Cochran and associates (2), and the cows were allotted to the groups in each trial in accordance with the plan outlined by them. The cows were all of the Holstein-Friesian breed, and each had one or more previous

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lactations. With five slightly deviating exceptions, the experimental periods began at least one month after calving and ended not later than the eighteenth week of pregnancy. The equalized-feeding method devised by Lucas (14) was followed with some modification due to the group feeding of roughage. When not being milked, the cows were in vegetation-free paddocks where chopped Napier grass was fed. Because the number of paddocks available was limited, the cows receiving a given concentrate mixture were placed together; thus only the total roughage consumption of the cows receiving a given mixture could be controlled and measured.

TABLE 1

The rations

Composition figures are from actual analyses. (All figures in percentages)

Concentrate mixture	Trials I and II			Trial III		
	Basal	Basal plus urea	Control	2.5% urea	1.25% urea	Control
Cane molasses	25.0	25.0	25.0	25.0	25.0	25.0
Pineapple bran	30.0	27.5	30.0	36.5	36.5	36.5
Soybean oil meal	3.5	3.5	21.0	4.0	12.75	21.5
Corn or barley	39.5	39.5	22.0			
Barley				30.0	22.5	15.0
Urea (Uramon)		2.5		2.5	1.25	
Salt	1.0	1.0	1.0	1.0	1.0	1.0
Bone char	1.0	1.0	1.0	1.0	1.0	1.0
Total crude protein equivalent	7.4	13.2	13.4	12.8	12.2	12.6
Total digestible nutrients	66.8	64.6	65.7	61.1	61.1	64.2
Ether extract	1.5	1.3	1.8	0.9	1.2	1.6

	Trial IV			
	No urea		Urea	
	Molasses	No molasses	Molasses	No molasses
Cane molasses	25.0		25.0	
Pineapple bran	30.0	55.0	28.0	53.0
Soybean oil meal	21.0	22.0	7.0	8.0
Barley	22.0	21.0	36.0	35.0
Urea (Uramon)			2.0	2.0
Salt	1.0	1.0	1.0	1.0
Bone char	1.0	1.0	1.0	1.0
Total crude protein equivalent	13.5	13.7	14.0	12.7
Total digestible nutrients	71.6	74.4	70.8	72.7
Ether extract	3.6	3.1	3.1	2.8

The concentrate mixtures, shown in table 1, were typical of those fed by dairymen in the Territory in that they contained large amounts of cane molasses and pineapple bran. The latter feed, also known as dried pineapple pulp, is a by-product of the pineapple industry. It consists of the pulp remaining after juice is extracted from the fruit, and also of the outer shell and the core. The soybean oil meal contained about 42 per cent total crude protein. The urea was a commercial product called "Uramon." In the initial trial, corn made up the cereal portion of the concentrate mixture. Since corn became unavailable later, barley was used throughout the remaining trials.

The Napier grass was fed in equal amounts to all lots in a given trial and in amounts such that practically all would be consumed. Concentrates were fed in quantities to supply considerably more total digestible nutrients than is recommended by Morrison (16) for good cows under usual conditions. Such heavy feeding is commonly practiced in the tropics and is undoubtedly justified because of the high price of milk in relation to feed costs (12) and because of the apparent need of cows in the tropics and subtropics for greater intakes of energy to maintain reasonable production. The total digestible protein in the control rations was calculated to supply somewhat more than Morrison recommends for good cows under usual conditions. The control and urea rations were computed to supply the same amounts of total crude protein equivalent. The term "protein equivalent" refers to the nitrogen in the ration multiplied by 6.25.

The chemical compositions of the feeds and the nutrient intake data presented are based upon analyses of the feeds in question. In calculating digestible nutrients from the proximate analyses, the coefficients for apparent digestibility given by Morrison (16) were used for soybean oil meal, corn, and barley. The coefficients determined by Work (20) were used for pineapple bran and Napier grass.

The milk yields have been converted to the same energy equivalent of 4 per cent fat-corrected milk (F.C.M.) by Gaines' formula (5). The data from the first week of each period were omitted to minimize carry-over effects from one period to another. Statistical analyses were carried out in accordance with the methods outlined by Cochran and associates (2) and Snedecor (19).

TRIALS I AND II. UREA PARTIALLY UTILIZED BY DAIRY COWS

The procedures in the first two trials were identical with two exceptions. In the first trial corn was used, and the three experimental periods were each three weeks in length. In the second trial barley replaced corn, and the length of each period was four weeks. The data have been combined and are presented together. The purpose of these trials was to determine if urea was utilized by cows receiving typical Hawaiian rations and if the production of cows receiving part of their nitrogen from urea would compare favorably with cows receiving all from plant sources.

Accordingly, at a given time two of the six cows in each trial received a basal ration which would supply inadequate protein. Two received the basal plus enough urea to raise the protein equivalent in the ration to the level required. Two received the control ration which consisted of the basal plus soybean oil meal and which contained required amounts of protein. The results are presented in table 2.

During the last period in the first trial one cow receiving the urea ration went "off feed" to such an extent that she had to be removed from the experiment. For statistical analysis the missing data for this cow were estimated. For convenience in statistical interpretation of the milk yields, the mean which included the estimated value is presented in the table. An estimated value for this cow is also included in the average for concentrate consumption. These means and those calculated without the estimated values were not essentially different.

TABLE 2

Results of trials I and II. Average daily milk yields and feed and nutrient intakes of cows receiving the basal (low protein) ration, the basal plus urea, and the control ration (basal plus soybean oil meal)

Ration	Basal	Basal plus urea	Control
Cow periods, No.	12	12	12
4% F.C.M. yields, lbs.	20.0	21.6	22.8
Concentrate consumed, lbs.	16.5	16.3	16.5
Napier grass consumed, lbs.	56.0	59.0	59.0
T.D.N. required, lbs.*	15.3	15.7	16.2
T.D.N. consumed, lbs.	17.9	17.8	18.2
Digestible protein required, lbs.*	1.70	1.77	1.84
Total non-urea digestible protein consumed, lbs.	1.09	1.03	1.99
Total crude protein equivalent consumed, lbs.	1.83	2.81	2.89
Urea total crude protein equivalent consumed, lbs.		1.08	
Urea consumed, lbs.		0.41	
Least difference between 4% F.C.M. means required for probability of			
5%		0.95	
1%		1.40	

* According to Morrison (16) for good cows under usual conditions.

There was a highly significant difference (probability less than 1 per cent) in 4 per cent F.C.M. yields of the cows when receiving the basal ration and when receiving either of the other two rations. These data demonstrated that urea was utilized for milk production. The difference in F.C.M. yields of the cows when receiving the urea and the control rations was significant (probability less than 5 per cent), indicating that the nitrogen from urea was not utilized as efficiently as that from soybean oil meal.

TRIAL III. COMPARISON OF RATIONS CONTAINING TWO LEVELS OF UREA WITH A NON-UREA RATION

In the two previous trials the urea ration contained 2.5 per cent urea in the concentrate mixture. This amount of urea supplied over one-third of the total intake of total crude protein equivalent. It was thought that this large amount might account for the lower production of the cows when receiving the urea ration than when receiving the control ration. A third trial with six cows was, therefore, planned to compare a ration containing 2.5 per cent urea in the concentrate mixture with one containing 1.25 per cent urea and with a control ration containing soybean oil meal as the only protein supplement. The experimental periods were four weeks in length. The results are presented in table 3.

TABLE 3

Results of trial III. Average daily milk yields and feed and nutrient intakes of cows receiving concentrate mixtures containing 2.5 per cent, 1.25 per cent and no urea (control ration)

Ration	2.5% urea	1.25% urea	Control
Cow periods, No.	6	6	6
4% F.C.M. yield, lbs.	26.5	27.1	28.9
Concentrate consumed, lbs.	19.1	18.9	19.0
Napier grass consumed, lbs.	63.0	63.0	62.0
T.D.N. required, lbs.	17.9	18.1	18.7
T.D.N. consumed, lbs.	19.8	19.7	20.2
Digestible protein required, lbs.	2.06	2.09	2.18
Total non-urea digestible protein consumed, lbs.	1.30	1.73	2.31
Total crude protein equivalent consumed, lbs	3.47	3.34	3.41
Urea total crude protein equivalent consumed, lbs.	1.26	0.63	
Urea consumed, lbs.	0.48	0.24	
Least difference required between 4% F.C.M. means for probability of			
5%		0.73	
1%		1.21	

Although there was no significant difference between the milk yields of the cows when receiving the two urea rations, there was a highly significant difference between their yields when receiving either of the two urea levels on the one hand and no urea on the other. Consequently, it would appear that the feeding of large amounts in the preceding trials was not the main cause of the low production when receiving urea.

TRIAL IV. EFFECT OF LARGE AMOUNTS OF MOLASSES ON UREA UTILIZATION

Since cane molasses made up 25 per cent of the concentrate mixtures in the previous experiments, it was thought such high levels might be the cause of the comparatively low milk production of the cows when receiving the urea rations. Rupel and associates (18) have shown that levels of corn molasses up to 10 per cent do not cause detrimental effects. Other work at the same station (15) has demonstrated, however, that molasses as the only source of readily available carbohydrate in the ration did not enable satisfactory urea utilization. The next experiment at the Hawaii Station was, therefore, designed to determine if the large amounts of molasses fed in Hawaii may, likewise, cause poor utilization even if other sources of carbohydrate are contained in the concentrate mixture.

Eight cows were divided into two groups: one to receive 25 per cent cane molasses in the concentrate mixture, and one to receive no molasses. Each of these two groups was, in turn, divided into two subgroups: one to receive 2 per cent urea in the concentrate mixture and the other none. This trial was, therefore, a factorial experiment. Each period was three weeks in length. The results are presented in table 4.

TABLE 4

Results of trial IV. Average daily milk yields and feed and nutrient intakes of cows receiving rations with and without urea and with and without molasses in a factorial experiment

Ration	No urea		Urea	
	Molasses	No molasses	Molasses	No molasses
Cow periods, No.	8	8	8	8
4% F.C.M. yield, lbs.	21.0	22.4	21.2	21.3
Concentrate consumed, lbs.	16.0	16.1	16.0	15.5
Napier grass consumed, lbs.	71.0	69.0	72.0	71.0
T.D.N. required, lbs.	14.8	15.3	14.9	14.9
T.D.N. consumed, lbs.	22.2	22.4	22.3	22.0
Digestible protein required, lbs.	1.69	1.76	1.69	1.70
Total non-urea digestible protein consumed, lbs.	2.07	2.07	1.42	1.22
Total crude protein equivalent consumed, lbs.	3.02	3.05	3.12	2.83
Urea total crude protein equivalent consumed, lbs.			0.84	0.82
Urea consumed, lbs.			0.32	0.31

When receiving the urea-no-molasses ration one cow refused part of her feed for over a week. She, nevertheless, continued to maintain her anticipated milk flow. This fact, along with the unaccountably low protein content of the concentrate mixture, accounts for the low average feed and protein intake of the cows when receiving this ration.

There were no significant differences in milk yields when the cows were receiving the different rations. Contrary to the expectation that the molasses might be detrimental to maximum urea utilization, the trend of the data was actually in the direction to indicate that molasses may promote the utilization of urea. It is interesting to note that the over-all trend was in favor of the non-urea rations when compared with the urea rations. This was in agreement with the previous trials.

DISCUSSION

The results obtained from these trials are in agreement with other data collected at this station. In two double-reversal trials previously mentioned, Henke and associates (11) obtained significantly higher milk production from control rations than from rations containing urea. In addition, Henke (8) has alternately in three-week periods fed a urea and a non-urea ration to a group of cows. At first 2.5 per cent urea was fed, and later 1.5 per cent. These studies extended over intervals of fifty-seven and fifty-one weeks, respectively. Cows were occasionally added to or removed from the group. When receiving the control rations the milk production was 6.7 per cent higher than when receiving the 2.5 per cent urea, and 1.7 per cent higher than when receiving the 1.5 per cent urea ration.

In the four trials reported in this paper, the differences in favor of the non-urea rations containing adequate protein are greater on a percentage basis than those reported by most other workers. It is interesting to note, however, that Archibald (1) when using the short period, double-reversal type of experiment obtained greater differences than when using the continuous, long-period type. Such results might suggest that cows may need some time to become adapted to the utilization of urea, probably through changes in the flora of the rumen, and that long-period trials would, therefore, give a better measure of its value. Most workers using the continuous type of trial, including Archibald (1), Rupel and associates (18), and Hastings (7), report slight differences. On the other hand, Ehrenburg and associates (3), also using a continuous-type trial, reported a difference of similar magnitude on a percentage basis and in the same direction as those obtained in the double-reversal trials conducted at the Hawaii station. Owen and associates (17), using periods of varying lengths, but with most being thirty-six days or less, obtained no appreciable difference. It is not at all conclusive, therefore, that long-period trials necessarily should give more reliable results than short-period ones, at least enough so to compensate for the greater precision in measuring small differences possible with the reversal type. The continuous trial of the nature of that run at Wisconsin (18) is, however, undoubtedly valuable in determining the effects of feeding urea throughout a complete lactation.

In the four trials reported in this paper the mean 4 per cent F.C.M. yield of the cows when receiving 2.0 or 2.5 per cent urea in the concentrate mixtures can be compared with the mean yield when receiving soybean oil meal as the only high-protein supplement at required levels. The probability of the difference being due to chance alone is less than one-tenth of 1 per cent as determined from the variance ratio tables of Fisher and Yates (4).

Because some feed refusals occurred during these trials, there were some differences in nutrient intakes between the treatments being compared. For this reason, the 4 per cent F.C.M. yields were adjusted to the same total crude protein equivalent and total nutrient intakes by means of covariance. These adjustments made no appreciable changes in the results or in their interpretation. In these analyses an accurate regression coefficient for milk yield on nutrient intake could not, however, be determined, for the practice of holding the cows receiving a given concentrate mixture in one paddock where the roughage was fed, did not permit a measure of individual roughage consumption. Only average figures for each paddock were used. Adjusting the milk yields for carry-over effects from one period to another according to the method outlined by Cochran and associates (2) also made no appreciable changes in the results.

The rations fed in these trials are, as previously mentioned, typical of those fed in the Territory. The fat contents of the concentrate mixtures, as can be seen by inspection of table 1, were in most cases below 2 per cent, considerably lower than the amounts found necessary for maximum production in the Cornell studies (13). Limited work by Henke and associates (9, 10) has indicated that an increase in 4 per cent F.C.M. production may result from adding fat to low-fat rations similar to those used as controls in these trials. The fat contents of the rations also decreased with the addition of urea. It is, therefore, possible that the effect of differences in fat contents was being measured as well as the effect of urea feeding. In urea studies with similar rations but with the fat adjusted close to 4 per cent by use of coconut oil meal, Henke and associates (11), nevertheless, obtained differences which were significant but of a lower magnitude on a percentage basis than those reported in this paper. To determine the part that fat has played in the results of these urea studies will require further investigation. There is always the possibility that conditions peculiar to Hawaii, such as the all-year-round feeding of low-protein soilage crops or the climate, may directly or indirectly be responsible for the relatively poor results obtained when feeding urea at this station.

Although, as mentioned previously, two cows had digestive disturbances when receiving the urea rations, there were feed refusals by cows receiving other feeds as well; so it cannot be definitely said that urea would cause more trouble in this respect than if it had not been fed.

In any event, at least under Hawaiian conditions, the dairyman apparently must expect some drop in milk production when urea on an equal total crude protein equivalent basis is substituted for part of the high-protein supplement in the ration. This fact, along with the relative costs of feeds, must, then, be taken into consideration when determining the economy of feeding urea. One could not afford to feed it unless cereal grains or other high-energy feeds are considerably cheaper than high-protein feeds or unless the latter are unavailable.

SUMMARY

Four double change-over urea-feeding trials involving twenty-six Holstein-Friesian cows in eighty-six cow-periods are described. The conclusions are as follows:

1. The dairy cow is able to utilize nitrogen from urea in the production of milk, but apparently not as efficiently as the nitrogen derived entirely from plant sources.

2. Less milk was obtained when urea was fed whether the daily intake was 0.48 pound or 0.24 pound. These amounts provided 36 per cent and 19 per cent, respectively, of the total crude protein equivalent intake.

3. The feeding of cane molasses in amounts to make up 25 per cent of the concentrate mixture had no detrimental effect upon the synthesis of protein from urea in the rumen.

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MOISTURE STUDIES IN DRY PRODUCTS OF MILK. I. KINETICS OF THE DEHYDRATION OF ALPHA LACTOSE HYDRATE IN TOLUENE

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In connection with our studies on the toluol distillation method of determining moisture in dry milk products (1), we had occasions to determine the moisture desorption-time curve of pure lactose monohydrate. It was found that the rate of moisture removal follows quite well the unimolecular kinetics expression,

$$k = \frac{1}{t} \ln \frac{a}{(a-x)}$$

where "k" is the rate constant; "a," the initial moisture content (5.0%); and "x" the amount of water removed in time "t." As lactose monohydrate is the most difficult constituent in dry milk products to dehydrate (3), it was thought interesting to determine the magnitude of the rate constant and to study some of the factors, such as particle size and rate of distillation, which affect its rate of dehydration, since the results of such a study might throw some light on the conditions necessary for quantitative removal of water from dry milk products by the toluol distillation method.

EXPERIMENTAL PROCEDURE

The apparatus, which consists of a 300-ml. Erlenmeyer flask, a Liebig condenser, a Precision electric heater (type RH), and a moisture trap graduated from 0 to 4 ml. in 0.1-ml. divisions, was the same as that used in this laboratory for the routine determination of moisture (1), except that a motor-driven and mercury-sealed stirrer was inserted to help agitate the sample. The toluene used was of C.P. grade with zero blank for moisture. The lactose monohydrate was Baker's C.P. powder, containing 5.0% water as determined by the Karl Fischer method (4).

Fifty grams of lactose monohydrate of the desired particle size as determined by U. S. Standard sieves were weighed into a 300-ml. Erlenmeyer flask and quickly covered with 100 ml. of toluene. After attaching the flask with sample to the apparatus, enough toluene was added through the top of the condenser to fill the moisture trap. Stirring was then started at a rate of about 3,400 r.p.m. The rate of condensation was adjusted to give two to three drops per second. After the first appearance of moisture in the trap, the moisture collected was read at several time intervals. This was multiplied by two to convert to per cent of moisture desorbed. Droplets which failed to settle out were dislodged by means of a stiff long wire prior

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to each reading. The logarithm of the difference between the initial moisture, which is 5.0 per cent, and that determined at each time interval was then plotted against the corresponding time in minutes. The slope of the best straight line through the points, when multiplied by the conversion factor 2.303 gives the rate constant per minute.

RESULTS AND DISCUSSION

In figure 1 are shown some of the dehydration curves of different particle sizes of lactose monohydrate. As might be expected from the general influence of surface area on the rate of evaporation, the effect of decrease in particle size is to increase the rate of dehydration. Furthermore, by assuming the particles to be spherical and the rate constant to be a function of the total surface area, which per unit weight, is the product of the number of particles and the surface area per particle, it can be shown mathematically that the rate constant "*k*" varies inversely with the first power of the average diameter "*d*." The quantity "*d*" is calculated by taking the arithmetical mean of the lowest and the highest sieve sizes of each series. The

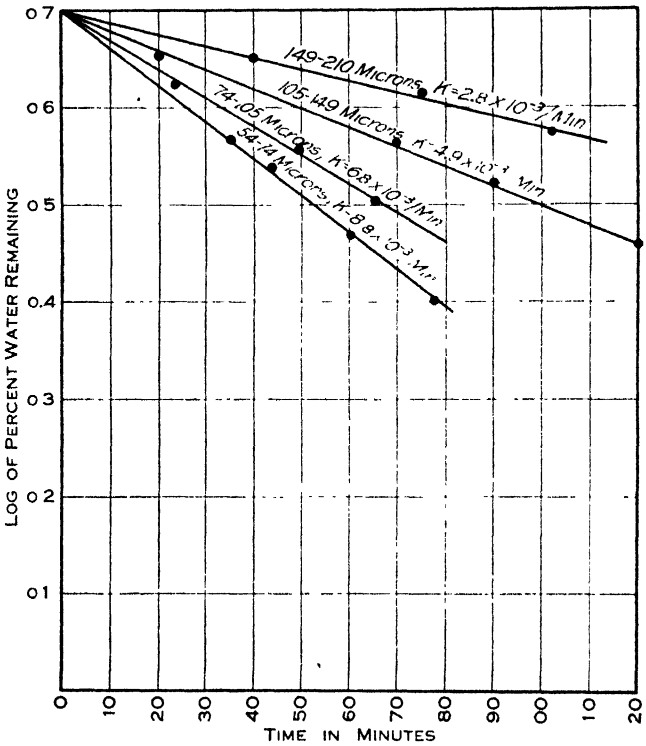

FIG. 1. Rates of dehydration of lactose monohydrate of different particle sizes.

experimental data, when plotted as in figure 2 gives rise to a straight line, expressed by the equation, $k = \frac{0.565}{d}$, in excellent agreement with the above calculation.

The magnitude of the rate constants is also interesting. Since the reaction is unimolecular, the rate constant has the dimension per unit time. Therefore at a rate constant of 2.8×10^{-3}/min., or 0.168/hour, as in the case of particle sizes 149–210 microns, 16.8 per cent of the total amount of lactose hydrate present at any given time is dehydrated per hour. For particle sizes 54–74 microns, 53 per cent of the total lactose monohydrate is dehydrated per hour. It is apparent that an adequate toluol distillation time for the determination of moisture in dry products of milk is dependent upon the particle size and the amount of lactose hydrate, and thus cannot be arbitrarily established. For this reason the method of the American Dry Milk Institute (1) provides for one hour distillation and additional fifteen-minute distillation periods until constant moisture values over a fifteen-minute period are obtained. The slow rate of dehydration necessitates three hours distillation time for the quantitative determination of moisture in most dry whey solids, and may also explain why moisture results for some dry milk products obtained by the toluol distillation method, using one hour distillation are lower than those obtained by the Karl Fischer method as found in this Laboratory and by Heinemann (2) in his recent paper.

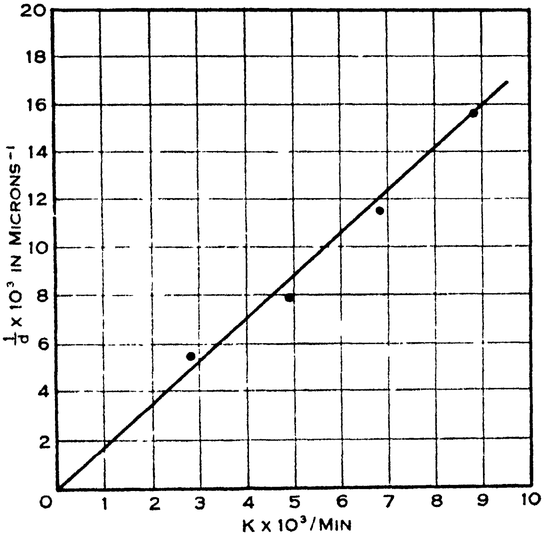

FIG. 2. Relation of particle diameter to the rate constant for the dehydration of lactose monohydrate.

The effect of distillation rate on the rate of dehydration was next studied with the aim of finding whether there exists a rate of distillation above which the velocity of dehydration is constant. Results of such studies, using lactose monohydrate of particle sizes under 149 microns in diameter, are shown in figure 3. The last three points of the curve taken in the order of increasing distillation rate represent the maximum temperature of the hot plate (about 400° C.) in combination with stirring rates of 3,400, 4,400 and 5,800 r.p.m., respectively. It can be seen that above a distillation rate of 6 ml. per minute, or approximately 2 drops per second, the velocity of dehydration is independent of the rate of distillation. This result indicates that the rate of 3 drops per second as specified by the American Dry Milk Institute (1) for the determination of moisture in dry milk products is adequate.

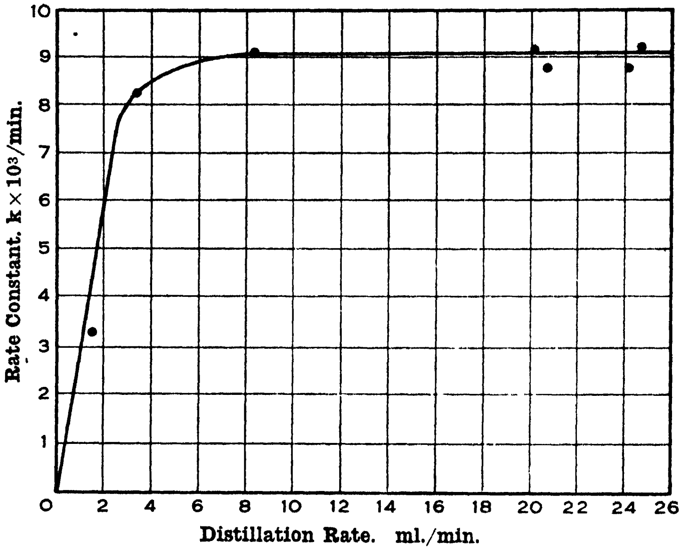

FIG. 3. Effect of distillation rate on the velocity of dehydration of lactose monohydrate.

The results of this investigation may possibly be applied to the determination of lactose hydrate in some dry milk products such as dry whey, since, in these products, surface moisture and moisture bound by proteins are easily desorbed (3), so that a logarithmic plot of the total moisture present at any time "t" against the time should theoretically result in a curve composed of two distinct portions. The first steeper portion represents the dehydration of lactose hydrate and the other loosely bound forms of moisture, while the second portion which should be linear, represents the dehydration of lactose monohydrate alone. Therefore, extrapolation of the second portion of the curve to zero time will give the initial amount of water bound as the hydrate of lactose. This value when divided by 0.050 should yield the per cent of lactose monohydrate in the sample. This possibility is being investigated.

SUMMARY

The dehydration of lactose monohydrate in boiling toluene is found to be a first order reaction, the rate constant of which varies with particle size in accordance with the equation $kd = 0.565$, where "k" is the rate constant in min.^{-1} and "d" is the average diameter of the particle in microns. The effect of the magnitude of the rate constant on the length of distillation period necessary for the quantitative determination of moisture for dry milk products by the toluol distillation method is discussed. The rate of distillation has some influence on the velocity of dehydration of lactose monohydrate, but above a distillation rate of 6 ml. per minute any further increase in the distillation rate has no effect on the rate of dehydration.

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COLORIMETRIC DETERMINATION OF AMMONIA IN MILK AND DRY PRODUCTS OF MILK

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In the course of our study of proteolysis in dry products of milk, we needed a rapid and sensitive method for determining ammonia. The small amount of ammonia present in fresh fluid milk and in dry milk products makes accurate determination a matter of some difficulty. The various methods have been reviewed by Perkins (3), many of which leave much to be desired from the standpoints of accuracy and convenience. Preliminary experiments in this Laboratory showed that the permutit adsorption method of Folin and Bell (2) does not give satisfactory recovery of added ammonia either in milk or in deproteinized milk filtrate. This was also found to be true by Perkins (3). Nessler's reagent is too sensitive to volatile constituents from rubber when using steam distillation. However, the reactions of ammonia with alkaline phenol and sodium hypochlorite solution have been used by Van Slyke and Hiller (6) and by Borsook (1) with some success. Recently, Russell (5) has improved the sensitivity and reproducibility of the method with the result that as little as 0.5 microgram of ammonia may be conveniently determined. This improved method forms the basis of the following procedure in which milk is deproteinized with sodium tungstate in sulfuric acid solution and the filtrate steam distilled with magnesium hydroxide to liberate the ammonia.

Reagents: 1. Sodium Tungstate (10%). 100 grams of sodium tungstate are dissolved in 900 ml. of distilled water.

2. Sulfuric Acid ($\frac{1}{2}$ N). To a liter volumetric flask containing about 300 ml. of water, 13.6 ml. of concentrated sulfuric acid low in ammonia are added and the volume diluted to 1 liter.

3. Sulfuric Acid (0.01 N). One part by volume of 0.5 N H_2SO_4 is diluted with 49 parts by volume of water.

4. Magnesium Hydroxide (8%). Eighty grams of magnesium oxide (C.P.) are stirred into 600 ml. of water. The content is boiled for 5 minutes. After cooling the solution is made up to 1 liter.

5. Alkaline Phenol Reagent. Twenty-five grams of crystalline phenol are mixed with a small amount of water. To this 54 ml. of 5 N sodium hydroxide are added with stirring. After making up to 100 ml., the solution is preserved in a brown bottle at refrigeration temperature.

6. Hypochlorite Solution. Twenty-five grams of calcium hypochlorite (U.S.P.) are dissolved in 300 ml. of hot water. To this, 135 ml. of potassium carbonate solution (20%) are added. The mixture is heated to 90° C., cooled,

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and made up to 500 ml. A small volume of solution is filtered and the filtrate examined for Ca++ by adding more potassium carbonate solution. If a positive test is obtained more carbonate is added until no Ca++ is present in the filtrate. The mixture is then filtered and stored in small brown bottles in the refrigerator. The solution must be water clear and contains from 1.3 to .4 grams of free chlorine per 100 ml. as determined by iodometric titration. The strength of this reagent is checked occasionally.

7. Managanese Sulfate (0.003 M).

Procedure: Ten grams of the dry milk product are added to 93 ml. of distilled water and shaken vigorously to bring the solids into uniform colloidal suspension. The final volume is close to 100 ml., since the partial specific volume of all dry products of milk is about 0.7 ml. Ten milliliters of this reconstituted milk are taken for analysis. The proteins are precipitated by means of 10 ml. of 10 per cent sodium tungstate and 20 ml. of $\frac{1}{2}$ N sulfuric acid, added in the order named. A twenty-five milliliter portion of the filtrate representing 0.625 grams of the sample, or a smaller aliquot if the sample is high in ammonia, is steam distilled in a Pregl apparatus, using 10 ml. of 8 per cent magnesium hydroxide to liberate the ammonia, 5 ml. of 0.01 N sulfuric acid as the absorbing agent, and a distilling time of seven minutes.

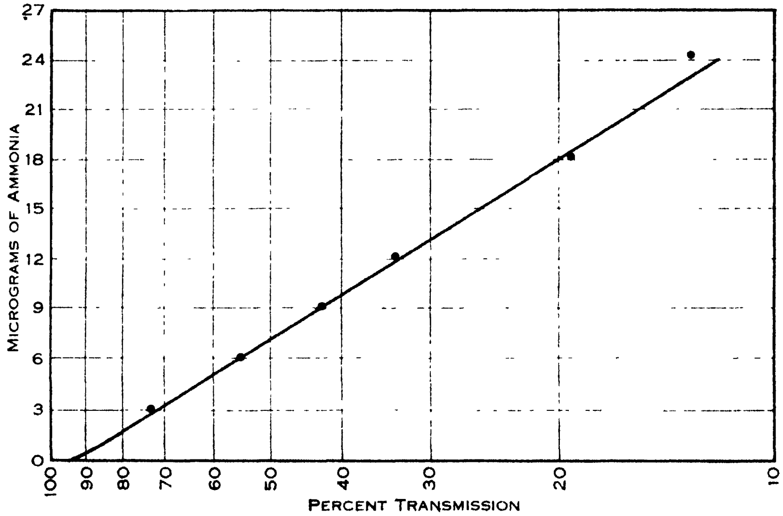

FIG. 1. Calibration curve.

The distillate is transferred to a 50 ml. volumetric flask and diluted to the mark. An aliquot of 1.0 to 5.0 ml. containing from 2 to 20 micrograms of ammonia is pipetted into a 15-ml. centrifuge tube kept in an ice bath. A drop of manganese salt solution is added to the sample, followed by 1 ml. of phenol reagent and 1 ml. of hypochlorite solution. After gently mixing the content by means of a stirring rod, the tube is immediately placed in a boiling water bath for 5 minutes. The sample is removed from the bath, stirred vigorously, and allowed to cool. When cooled, the volume is made up to 10 ml. The blue color developed is read in a Pfaltz and Bauer photoelectric colorimeter, using matched test tubes and a filter having maximum absorption at 6100 Å. The quantity of ammonia corresponding to the observed transmission is estimated from a curve (Fig. 1), previously calibrated with varying concentrations of ammonium sulfate. A blank containing equivalent amounts of all reagents is also run. The total amount of ammonia calculated in milligrams per 100 grams of sample is obtained by means of the following expression:

$$\frac{100V_0'Y}{WV_x} = \text{mg. ammonia/100 grams}$$

V_0' = total volume of distillate
V_x = volume of distillate taken for color development
Y = (mg. ammonia in sample) – (mg. ammonia in blank)
W = Grams of sample by aliquot of filtrate taken for distillation.

RESULTS AND DISCUSSION

Steam distillation has been successfully employed in many micro-Kjeldahl procedures for isolating ammonia, and has the advantage of a short distillation time. Since in this procedure a weaker base, magnesium hydroxide, is used instead of the usual sodium or potassium hydroxide, it was necessary to determine the time of distillation needed for quantitative removal of ammonia from the deproteinized filtrate. Results summarized in table 1 for a sample of dry whey solids using a 10 ml. aliquot, and similar data on other dry products of milk not shown here, indicate 7 minutes distillation to be sufficient. It may be pointed out that the dry whey sample used above has the highest ammonia content yet encountered and that the 10 ml. aliquot of the deproteinized filtrate contains almost 500 micrograms of ammonia, an amount seldom found in even a 25 ml. aliquot from most dry products of milk.

Several recovery experiments were also run by adding known quantities of ammonia to a sample of reconstituted dry buttermilk. Results presented in table 2 show that over 95% of the added ammonia was recovered in each case.

TABLE 1

Effect of varying the time of distillation on the amount of ammonia liberated

Distillation time (Mins.)	NH_3 liberated (Mgs./100 gms.)
3	149
5	197
7	198
8	198

TABLE 2

Recovery of ammonia added to reconstituted dry buttermilk

NH_3 added (Micrograms/ml.)	NH_3 found (Micrograms/ml.)	Recovery of added NH_3 (Micrograms/ml.)
0	4.5	0
5.0	9.7	5.2
10.0	14.4	9.9
15.0	19.9	15.4
30.0	33.5	29.0

Several dry products of milk were examined for ammonia by the above procedure. Of the large number of each analyzed, dry whole milk contained from 3 to 7 mgs./100 gms. and nonfat dry milk solids from 4 to 9 mgs./100 gms. The ammonia content of dry buttermilk solids ranged from 3 to 82 mgs./100 gms. with most of the samples containing less than 15 mgs./100 gms. Dry whey solids, in general, shows high ammonia content, the range being from 20 to 198 mgs./100 gms. Of the several samples of commercial fluid whole milk examined, the ammonia varies from 0.30 to 1.30 mgs./100 ml., values which are of the same order of magnitude as those reported by previous workers (3, 4).

In order to gain some ideas about the reproducibility of the procedure, ten separate determinations were run on the same sample of pasteurized milk. The data are presented in table 3. A statistical analysis of the results yielded an average deviation from the mean of 0.016 mg./100 ml. and a standard deviation of 0.026 mg./100 ml., indicating satisfactory reproducibility.

TABLE 3

Reproducibility of the method

Trial	Mg. NH_3/100 ml. milk	Deviation (d) from average
1	0.66	0.04
2	0.70	0.00
3	0.70	0.00
4	0.75	0.05
5	0.70	0.00
6	0.70	0.00
7	0.73	0.03
8	0.66	0.00
9	0.70	0.00
10	0.70	0.00
Average	0.70	0.016

Standard Deviation = 0.026 mg./100ml.

SUMMARY

A colorimetric method is described for the determination of ammonia in fluid milk and in dry products of milk based upon the reaction of ammonia with alkaline phenol and sodium hypochlorite. A statistical analysis of

data for one sample of fluid milk shows an average deviation from the mean of 0.016 mg./100 ml. and a standard deviation of 0.026 mg./100 ml., indicating satisfactory reproducibility.

The ammonia content of some dry milk products and fluid milk varies considerably with dry whey solids having the highest ammonia content.

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THE RELATIONSHIP OF THE GROWTH OF ALL BACTERIA AND COLIFORM BACTERIA IN PASTEURIZED MILK HELD AT REFRIGERATION TEMPERATURES*

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A recent study on the keeping quality of pasteurized milk has been published by Dahlberg (1, 2) in which total bacterial counts and coliform counts were made on 108 different lots of pasteurized milk collected in July–August, 1945, and October, 1944. The pasteurized milk was obtained from six milk companies in the New York Metropolitan area that processed about 800,000 quarts daily. The milk was held for four days at three different refrigeration temperatures. The bacterial counts were made on standard agar and desoxycholate agar using the procedure outlined in the 1941 edition of *Standard Methods for the Examination of Dairy Products,* published by the American Public Health Association. The data gave an opportunity to compare the rate of growth of all bacteria and coliform bacteria as they occur in a pasteurized milk supply of excellent quality.

It has been customary to teach that the lactic acid bacteria grow more rapidly than the coliform bacteria at cold temperatures. However, in raw milk Sherman (3) found that "our studies at various temperatures have shown that the colon count in milk generally increases more rapidly than the total count during the presouring stage, which includes the whole period of interest in the fluid milk industry." The temperatures employed were 50°, 59°, 70° and 86° F. Morris (4) found that coliform cultures grew more rapidly in pasteurized than in raw milk due to the bactericidal substance naturally occurring in raw milk.

RESULTS

The data, table 1, include all milks for total bacterial counts and each count represents 54 different bottles of milk. The coliform counts were averaged for those milks which were coliform positive so the initial counts in October include only about 6 per cent of the samples and in July–August about 35 per cent. However, after storage at 55–60° F. for 4 days from 73 to 88 per cent of the milks were coliform positive. If all milks had been included irrespective of the presence of coliform bacteria, all coliform counts would have been lower.

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* Acknowledgments: Several of the leading milk companies in the New York–New Jersey Metropolitan area assisted in this study by providing the required samples of milk, by providing necessary laboratory facilities, and through research grants to Cornell University. The author is indebted to Eleanor Brusten, Marion Goldberg, Eva Schiffer, and Maria Gonzalez for making the bacteriological analyses.

TABLE 1

The total bacterial count and the coliform count on 108 lots of pasteurized milk in 1620 quart bottles collected at 6 New York Metropolitan plants on 3 consecutive weeks in July–August and in October, and stored at 3 different temperatures

Days stored	Bacterial counts *					
	35–40° F.		45–50° F.		55–60° F.	
	Total	Coliform	Total	Coliform	Total	Coliform
October						
0	14,000	2	13,000	3	13,000	2
1	12,000	1	12,000	3	11,000	3
2	12,000	2	13,000	19	133,000	3,000
3	8,000	2	16,000	397	2,100,000	373,000
4	9,000	4	180,000	10,000	34,900,000	1,870,000
July–August						
0	13,000	3	15,000	2	19,000	5
1	18,000	5	19,000	10	77,000	343
2	14,000	11	27,000	57	444,000	66,000
3	13,000	9	84,000	4,000	5,400,000	565,000
4	11,000	123	335,000	295,000	11,900,000	6,000,000

* The total bacterial count was the standard plate count and the coliform count was determined in desoxycholate agar according to the 1941 edition of Standard Methods for the Examination of Dairy Products published by the American Public Health Association.

It will be noted, table 1, that the total bacterial counts tended to decrease during storage for 4 days at 35–40° F., but coliform counts at 35–40° F. remained constant in cool weather and actually increased slightly in warm

TABLE 2

The growth of all bacteria and coliform bacteria in pasteurized milk expressed as percentages of the total count that were coliform bacteria

Days stored	Percentages of total counts that were coliform		
	35–40° F.	45–50° F.	55–60° F.
October			
0	0.01	0.02	0.01
1	0.01	0.02	0.02
2	0.01	0.14	2.25
3	0.02	2.48	17.76
4	0.03	5.55	5.36
July–August			
0	0.02	0.01	0.02
1	0.03	0.05	0.44
2	0.08	0.21	14.86
3	0.07	4.76	10.27
4	1.12	88.05	50.42

weather. There was no increase in the percentages of all bacteria which were coliform types in cool weather but the percentages increased from 0.02 to 1.12 in warm weather, as shown in table 2.

At 45–50° F. the coliform bacteria increased more rapidly than the total count. After 4 days the coliform bacteria represented 5.55 per cent of the total count in October and 88 per cent in July and August. The much greater relative growth of coliform bacteria at 45–50° F. is illustrated by figure 1. The actual numbers of coliform bacteria were less than a half million per ml. after 4 days.

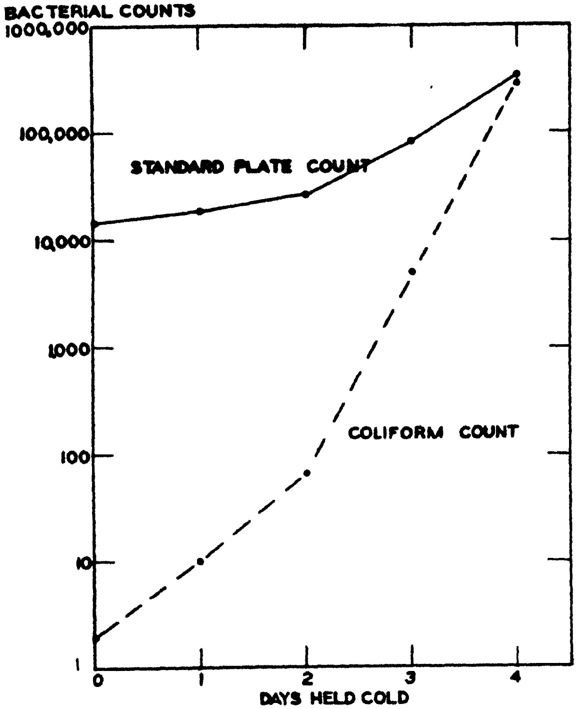

FIG. 1. The standard plate count for total bacteria and the coliform bacteria count on desoxycholate agar on 54 lots of milk collected in July–August and stored at 45–50° F.

The growth of coliform and all bacteria was rapid at 55–60° F. and even the number of coliform bacteria exceeded a million after 4 days. At this temperature bacteria other than coliform grew rapidly also. The coliform bacteria finally represented 5.36 per cent of the total bacteria in October and 50.42 per cent in July and August.

At all temperatures of storage the growth of coliform bacteria was more rapid in the warm summer months than it was during the cool month of October. This was true of total numbers of coliform bacteria and of the percentage of the total bacteria which were coliform.

DISCUSSION

As this study progressed it was observed that most of the bottles of milk which had been stored at 55–60° F. for 4 or 7 days showed gas in the cream layer. Sometimes in coagulated milk the gas was also evident in the skim milk. This observation substantiated the high coliform count obtained in milk held at so warm a temperature. It has been known for years that pasteurized milk often does not give a good lactic acid fermentation. The rapid growth of coliform bacteria, especially at 45–50° F., was a contributing factor toward abnormal fermentation as other bacteria grew so slowly at this temperature.

There are two possible explanations for the more rapid growth of coliform bacteria in pasteurized milk. It may be that under exactly comparable conditions the coliform bacteria multiply more rapidly than all other types of bacteria present in milk. In pasteurized milk, however, the coliform bacteria present are recontaminants which have not been subjected to the heat treatment and which are actively growing bacteria, especially in warm weather. All other bacteria in pasteurized milk are dormant as they have been subjected to refrigeration in the raw milk and growing strains have been destroyed by pasteurization.

The growth of coliform bacteria at all refrigeration temperatures including slight growth at 35–40° F. emphasizes the importance of making coliform tests only on freshly pasteurized milk if such tests are to be interpreted as indicating recontamination after pasteurization. Furthermore, such tests should be made promptly on well-iced samples. These facts are recognized but not always adhered to.

SUMMARY

The coliform bacteria in pasteurized market milk held at refrigeration temperatures increased more rapidly in numbers than the total count. In the freshly pasteurized milk less than 0.02 per cent of the total bacteria were coliform types. At 35–40° F. the percentage of the total bacteria which were coliform did not increase in October, but in July and August increased to 1.12 per cent in 4 days. After storage for 4 days at 45–50° F. and at 55–60° F. the coliform bacteria constituted about 5 per cent of the total count in October. During July and August the coliform count became 88 per cent of the total count after 4 days' storage at 45-50° F. and 50 per cent at 55–60° F. The coliform bacteria grew more rapidly in warm weather than in cool weather.

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THE EFFECT OF PREGNANCY ON THE BODY WEIGHT OF DAIRY COWS*

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Growth curves have been developed for all of the principal dairy breeds of cattle. Growth is usually measured by the height at withers and the weight of the animals. While the height at withers is not affected greatly by pregnancy, the weight of the animal may increase considerably. After a heifer is bred the weight curve takes an upward turn from the non-pregnant growth curve and is no longer a measure of growth alone but includes also the weight of the fetus and accompanying material including the fetal membrane and the amniotic fluid. After the heifer freshens, the weight curve declines to a new basic level and for a short period may be slightly below this level, especially if the cow is a heavy producer. The cycle is repeated for each pregnancy with the weight curve attaining a higher basic level until the cow is mature and this level becomes static.

Several studies (1, 2, 3, 4) have been made to determine how much of the gain during gestation is due to growth and how much can be ascribed to pregnancy, and to correct the growth curve which is turned awry when the animal becomes pregnant. The results of most of these studies, however, were based on small numbers of cows with consecutive pregnancies. All of these studies showed increases in body weights, month to month, from service to parturition, with the weight curves showing rather sharp upturns toward the middle and latter parts of the gestation period and at parturition the curves took sudden drops.

Bartlett (1) studied the increase due to pregnancy of grade dairy Shorthorn cattle over a five-year period, using 16 animals as the maximum number. He found that there was a progressive gain in weight, month by month, with a sharp upturn toward the end. The loss of weight at parturition varied from 154 to 190 pounds with an average of 170 pounds or 12.2 per cent loss of weight.

Moseley, Stuart, and Graves (3) took weights on 53 Holstein cows at an average age of 5 years and 3 months. There was an increase of weight from service to parturition of 285 pounds with the weight curve showing a sharp upturn during the middle and latter part of the gestation period. Ninety-four per cent of the gain occurred during the last five months of the period. The average weight of the calves was 97 pounds and the weight of the placenta and amniotic fluid was 58 lbs.

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Morgan and Davis (2) made the most extensive study so far reported. This involved all breeds and 656 separate gestations. They found that there was a progressive increase in weight from conception to parturition. During the first gestation these gains were approximately equal each month throughout this entire gestation period whereas during succeeding gestations the monthly gains proceeded rather evenly for the first four months only, and then the curve of gain started to bend upward and continued thus until parturition. The gain in weight during the first pregnancy was greater than in succeeding pregnancies for each breed and was approximately ⅓ the initial weight of the animal. For succeeding pregnancies the gain was only about ⅐ the weight of the cow. The loss due to calving was about ⅛ the initial weight of the cow, irrespective of age.

Willard (4) has given a table for Holstein heifers which includes additions to be added to the normal weight in calculating weight for pregnant animals. He took the differences between the weight of animals before and after calving as representing the gains during pregnancy.

EXPERIMENTAL

At the West Virginia Agricultural Experiment Station a study was made of the actual body weights (taken monthly) of 56 normal Ayrshire females during their first three pregnancies. The average age at first calving was 32 months; at the time of second calving, 46 months; and at the third calving, 59 months.

Table 1 shows the mean weights by months for the three different pregnancies. This table shows that there was a progressive gain in weight from pregnancy to parturition with a sharp decline at pregnancy.

The animals increased about 34.5 per cent of their initial weight during the first pregnancy; 20.5 per cent during the second pregnancy; and 19.0 per cent during the third pregnancy.

The average monthly gains and total gain for the period during each of

TABLE 1

Average body weights of 56 Aryshire females during pregnancy

Month of pregnancy	First pregnancy	Second pregnancy	Third pregnancy
	wt. lbs.	*wt. lbs.*	*wt. lbs.*
Service date	810	1003	1068
1	840	1003	1075
2	861	1014	1079
3	883	1028	1085
4	912	1042	1096
5	950	1062	1122
6	987	1096	1156
7	1024	1138	1197
8	1057	1157	1224
9	1091	1206	1270
Month after calving	991	1078	1128

the three gestation periods are given in table 2. During the first gestation period the animals increased in weight at a faster and more uniform rate than in the later periods. Approximately 50 per cent of the gain was made during the first five months of the first pregnancy while in the second and third pregnancies gains of only 29 and 27 per cent, respectively, were made. The total gain during the first pregnancy was 281 pounds while the gains for the second and third pregnancies were 203 and 202 pounds, respectively. This difference in increase was probably due to the fact that the younger animals were growing faster during this period and also the fact that they were not lactating.

The average gain during the eighth month of pregnancy was less than in the months immediately preceding and following. In the older cows this might be explained through the management procedure of giving each cow a two-month dry period. Often the need arises to reduce feed consumption during the early part of the dry period. The difference in the first-calf heifers was probably not significant.

TABLE 2

Average monthly gains in body weight of 56 pregnant Aryshires

Month of pregnancy	First pregnancy gain		Second pregnancy gain		Third pregnancy gain	
	For month	For period	For month	For period	For month	For period
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	30	30	0	0	7	7
2	21	51	11	11	4	11
3	22	73	14	25	6	17
4	29	102	14	39	11	28
5	38	140	20	59	26	54
6	37	177	34	93	34	88
7	37	214	42	135	41	129
8	33	247	19	154	27	156
9	34	281	49	203	46	202

In order to determine the gain in weight due to pregnancy, a growth curve is necessary to indicate the growth of open animals in comparison with the curve of the pregnant animals. As open animals were not available, a curve was drawn using the average weights of the animals at one month before they were bred as the normal weight of the animals at that time. The weights of the pregnant animals were then plotted and the difference noted as shown in table 3 and in figure 1. When the gains, attributed to pregnancy only, were considered it was found that as heifers they actually did not make as much total gain as a result of pregnancy as they did when older; *i.e.*, only 142 pounds as compared to 148 pounds during the second pregnancy and 162 pounds during the third pregnancy. The weights of the calves for the first pregnancy averaged 70.4 pounds; for the second, 74.6 pounds; and for the third, 74.8 pounds.

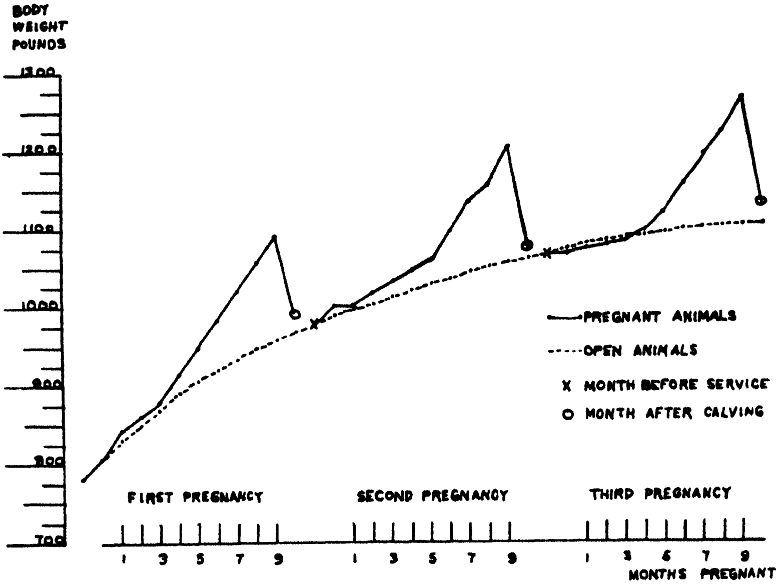

FIG. 1. Effect of pregnancy on body weight of Ayrshires.

DISCUSSION

The analysis of the data would indicate that approximately 50 per cent of the gain in weight of first-calf heifers can be attributed to permanent growth and the other 50 per cent to products of conception. The average permanent gain in weight for the first-calf heifers was about 16 pounds per month, the gain being faster during the early months of gestation. The slower growth of the animal during the latter months of pregnancy can be attributed to the larger nutritive demands of the fetus.

TABLE 3

Increase of body weights due to pregnancy

Month of pregnancy	First pregnancy			Second pregnancy			Third pregnancy		
	Gain per mo.	Total gain	Per cent inc.	Gain per mo.	Total gain	Per cent inc.	Gain per mo.	Total gain	Per cent inc.
	lbs.	*lbs.*		*lbs.*	*lbs.*		*lbs.*	*lbs.*	
1	10	10	1.2	4	4	0.4	−3	−3	−0.3
2	1	11	1.3	2	6	0.7	−1	−4	−0.4
3	3	14	1.7	6	12	1.3	1	−3	−0.3
4	11	25	3.1	6	18	1.8	7	4	0.4
5	21	46	5.7	12	30	3.0	22	26	2.7
6	21	67	8.3	27	57	5.7	30	56	5.3
7	23	90	11.1	35	92	9.3	37	93	8.8
8	20	110	13.5	13	105	10.6	25	118	11.1
9	32	142	17.5	43	148	14.9	44	162	15.7

The amount of weight which was ascribed to permanent growth during the second pregnancy was only about 27 per cent and for the third pregnancy, 20 per cent of the total gain in weight. In both the second and third this permanent gain in weight was evenly distributed throughout the gestation period. The gains attributed to conception were in general made during the middle and latter parts of the gestation period.

SUMMARY

1. A study was made of the effect of pregnancy on the body weights of 56 Ayrshire cows during the first three periods of gestation.
2. The body weights showed a definite pattern of increase as the stage of pregnancy advanced.
3. The gain in weight over and above normal weights was not great before the fifth month of pregnancy. From 75 per cent to 85 per cent of the gain came in the last four months of pregnancy.
4. The gain in weight from month to month was much more uniform with first-calf heifers than with the older animals. This was probably because the heifers were still growing and not producing milk.
5. The average monthly gains made during the eighth month of pregnancy was less than in the months immediately preceding and following.
6. The animals in the last month of pregnancy were on the average 151 pounds heavier than open heifers at the same age. During the first pregnancy they were 142 pounds heavier; during the second pregnancy, 148 pounds heavier; and during the third pregnancy, 162 pounds heavier.

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STUDIES ON THE CHEMICAL COMPOSITION OF CALF BLOOD*

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A study, conducted by the Department of Dairy Husbandry of this Experiment Station, pertaining to the raising of dairy herd replacements on dry calf rations, offered an excellent opportunity for obtaining a considerable amount of data concerning the values for several blood constituents of young calves. The components studied were carotene, ascorbic acid, nicotinic acid, calcium, phosphorus, glucose, cholesterol, and non-protein nitrogen; and the results of these analyses are presented in this report.

EXPERIMENTAL

Experimental animals. Guernsey, Jersey, and Holstein calves under five months of age were used in this study. They were maintained in individual pens for the duration of the experiment. Whole milk was fed in limited amounts for six to eight weeks, and a dry calf ration and mixed hay were added to the dietary as soon as the animals would consume them. The calves used in obtaining the following data made weight gains within the usual range and were apparently normal in all respects.

Methods of analysis. Carotene was determined spectrophotometrically at a wave-length of 450 mμ, after extraction as described by Yudkin (13), while ascorbic acid was measured according to the method of Mindlin and Butler (9). Non-protein nitrogen was determined by the method of Koch and McMeekin (8), sugar by the method of Folin and Wu (5), inorganic phosphorus according to Fiske and Subbarow (4), and analyses for calcium were carried out by the Clark-Collip procedure (3). Values for plasma cholesterol were obtained essentially as described by Sackett (11), while nicotinic acid determinations were carried out according to a method previously reported from the Chemical Laboratory of this Experiment Station (12) after extraction as described by Friedemann and Barborka (6). Final measurements of the above blood constituents, with the exception of carotene, which was determined spectrophotometrically, and calcium, which was determined titrimetrically, were carried out by use of a Klett-Summerson photoelectric colorimeter.

RESULTS AND DISCUSSION

A significant difference, with respect to plasma carotene was found be-

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[2] Department of Dairy Husbandry.

TABLE 1

Carotene and ascorbic acid values
(Holstein calves)

Constituents determined	Number of calves	Number of determinations	Amount in 100 ml. plasma *		
			High	Low	Average
Birth to 4 weeks					
Carotene	12	20	95.0	7.0	32.8
Ascorbic acid	12	18	0.73	0.22	0.41
5 to 8 weeks					
Carotene	12	21	37.0	10.0	20.2
Ascorbic acid	13	23	0.59	0.30	0.45
9 to 14 weeks					
Carotene	9	24	41.0	16.0	30.0
Ascorbic acid	9	23	0.66	0.26	0.42
15 to 23 weeks					
Carotene	11	26	82.0	16.0	44.0
Ascorbic acid	11	27	0.52	0.26	0.43

* Carotene expressed in micrograms; ascorbic acid expressed in milligrams.

tween results obtained with the Holstein calves and those obtained with the other two breeds. Furthermore, a preliminary study indicated a possible correlation between this factor and vitamin C. Therefore, two tables have been prepared to show the results for these two blood constituents, table 1 including the Holstein calves only and table 2 including the Jerseys and Guernseys. Since the remaining data did not show such a significant difference, the information presented in tables 3 and 4 represents results obtained with all of the animals used. As it seemed that the data would be more informative if they showed the values for different ages, these tables have been divided into four age ranges covering the period from birth to 23 weeks of age. No attempt has been made to show seasonal variations.

TABLE 2

Carotene and ascorbic acid values
(Jersey and Guernsey calves)

Constituents determined	Number of calves	Number of determinations	Amount in 100 ml. plasma *		
			High	Low	Average
Birth to 4 weeks					
Carotene	6	8	54.0	12.0	26.9
Ascorbic acid	5	7	0.64	0.30	0.40
5 to 8 weeks					
Carotene	13	17	74.0	8.0	36.0
Ascorbic acid	12	15	1.00	0.18	0.41
9 to 14 weeks					
Carotene	11	26	180.0	21.0	61.0
Ascorbic acid	11	27	0.99	0.16	0.45
15 to 23 weeks					
Carotene	12	37	144.0	21.0	64.5
Ascorbic acid	12	36	0.99	0.22	0.48

* Carotene expressed in micrograms; ascorbic acid expressed in milligrams.

However, it should be stated that the values shown resulted from analysis of blood samples collected during all seasons of the year.

In tables 1 and 2, values for carotene and ascorbic acid are presented. For these constituents the figures shown in the columns headed "high" and "low" are apparently true extremes, since such values were found in only a relatively small number of samples. The averages, as would be expected, show a much greater constancy, and also clearly show the considerably lower values obtained with the Holsteins, particularly for plasma carotene. From these average values, it is seen that the blood carotene increases but slightly, or even decreases, during the first few weeks, undoubtedly due to the using up of reserves furnished by colostrum, and then steadily increases as hay consumption increases. The ascorbic acid values show no particular correlation with age, the averages remaining quite constant. The values found were slightly higher than those previously reported by Bortree, Huffmann, and Duncan (1) but the finding of wide variations is in agreement with their results. Furthermore, it appears that breed differences with respect to this factor are not significant for calves of the age range studied.

TABLE 3

*Calcium, phosphorus, and glucose values**

Constituents determined	Number of calves	Number of determinations	High	Low	Average
Birth to 4 weeks			*mg.*	*per 100*	*ml.*
Calcium	12	21	13.0	10.9	12.12
Phosphorus	12	22	7.0	5.1	6.18
Glucose	11	20	150.0	77.7	110.00
5 to 8 weeks					
Calcium	17	24	12.8	10.0	11.68
Phosphorus	17	29	7.9	5.0	6.46
Glucose	16	28	113.0	60.8	89.70
9 to 14 weeks					
Calcium	12	23	12.0	9.7	11.03
Phosphorus	12	33	8.5	4.5	6.05
Glucose	12	30	93.5	64.3	75.70
15 to 23 weeks					
Calcium	15	38	12.6	9.8	11.03
Phosphorus	14	43	7.9	5.1	6.53
Glucose	15	44	86.0	60.0	74.20

* Calcium was determined on serum; phosphorus and glucose on whole blood.

There does not appear to be any correlation between age and blood calcium and phosphorus values (table 3), over the particular age range studied. The averages found for both of these constituents are, however, slightly higher than those reported for older animals by Kennedy, Anderson, Bechdel, and Shigley (7). Blood glucose, however, does show a definite decrease with age, and the data of table 3 upon which this statement is based agree exceedingly well with the findings of these workers (7).

The cholesterol values, seen in table 4, are of considerable interest since those of the greatest magnitude were found in the youngest age group, rather closely paralleling the results found for glucose, while the values found for the older calves are of the order of those which have been reported by Boyd (2) for cows. It is a rather well established fact that blood cholesterol values are affected by various factors; however, it is not yet possible to state the fundamental reason for such changes. Muller (10) in 1929 stated that the blood cholesterol level is "controlled by some substance which is present in varying amounts in animal organs." Possibly this "controlling factor" is of particular significance in the early life of the calf. In this same table are presented the values found for non-protein nitrogen and for nicotinic acid. The former, although slightly lower, are in good agreement with similar values found for older animals in a study previously cited (7). As for the nicotinic acid results, extensive data are lacking in the literature with which to make comparisons. Hence, these values, although resulting from too few determinations to warrant any definite conclusions, can, when supplemented by further investigations, be of considerable significance in the establishment of what might be considered the normal range of values for this vitamin in the blood of calves.

TABLE 4

*Cholesterol, nicotinic acid, and non-protein nitrogen values**

Constituents determined	Number of calves	Number of determinations	High	Low	Average
Birth to 4 weeks			*mg. per 100 ml.*		
Cholesterol (total)	14	24	180.0	87.0	130.0
Nicotinic acid	7	9	1.48	0.39	0.98
N. P. N.	11	20	42.4	23.0	29.40
5 to 8 weeks					
Cholesterol (total)	24	42	158.0	86.0	124.5
Nicotinic acid	14	21	1.42	0.19	0.81
N. P. N.	16	29	40.0	24.0	29.00
9 to 14 weeks					
Cholesterol (total)	20	49	186.0	71.0	108.3
Nicotinic acid	12	30	1.21	0.18	0.86
N. P. N.	12	30	36.7	22.0	31.40
15 to 23 weeks					
Cholesterol (total)	16	51	143.0	82.0	108.8
Nicotinic acid	9	14	1.26	0.40	0.79
N. P. N.	15	42	34.8	23.0	29.70

* Cholesterol was determined on plasma; nicotinic acid and NPN on whole blood.

SUMMARY

Data resulting from a large number of analyses for several blood constituents of normal Jersey, Guernsey, and Holstein calves have been presented. The results, tabulated in the form of ranges of values found, show what might be considered as normal values for calves of the age groups studied. An attempt was made, not to show the variations found in single animals, but rather to show average values for a fairly large number of animals. Correlation of the data with age has been made by presenting the results in four divisions, covering the period from birth to 23 weeks of age.

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THE EFFECT OF VITAMIN A DEFICIENCY ON REPRODUCTION IN DAIRY BULLS[1]

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Degenerative changes in the seminiferous tubules in laboratory animals resulting from vitamin A deficiency have been observed by numerous workers and reviewed by Mason (8). The need for vitamin A in maintaining normal reproductive functions in bulls is less well understood. Guilbert and Hart (3), Thorp and associates (15), Sutton and associates (14) and Erb and associates (2) have reported varying degrees of degeneration of the germinal epithelium in vitamin A deficient young bulls. With biopsies Moore (11) has shown varying degrees of damage of the germinal epithelium of young bulls when fed carotene at a level that just prevented blindness. A five- to six-fold increase in carotene intake promoted considerable repair of the damaged epithelium. Hart (4), Madsen and associates working in the Bureau of Animal Industry, United States Department of Agriculture (12), and Erb and associates (2) have reported that sperm production in vitamin A deficient bulls can be restored by adding this vitamin to the ration; this indicates that the damaged germinal tissue is reparable. Madsen and associates (12) observed that bulls of the beef breeds lose sex drive and produce semen of poor quality when developing avitaminosis A. Upon carotene therapy the breeding ability returned fairly rapidly but the quality of the semen improved more slowly. Jones and Haag (5) reported that two bulls made permanently blind as a result of vitamin A deficiency at six to eight months of age later in life produced semen that was apparently normal and one of them bred and probably settled three or four cows.

Additional information was needed on the actual fertility of bulls maintained on rations poor or deficient in vitamin A and to determine if an insufficiency of this vitamin might be a cause of low breeding efficiency. Furthermore there were no data available on the possible relation between various reproductive phenomena and atrophy of the anterior pituitary which frequently occurs in vitamin A deficiency in the bovine (Madsen *et al.*, 7). The present report gives data on the reproductive performance of dairy bulls raised and maintained on rations containing varying amounts

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of vitamin A and carotene that produced vitamin A deficiency at different ages; and of bulls maintained on rations containing different amounts of supplemental carotene after the development of typical vitamin A deficiency.

PROCEDURE

Six Holstein bulls, five Jersey bulls, and one crossbred Red Danish × Jersey bull were used in these experiments over the period from July 1941 to January 1946. Each bull was fed colostrum for 3 days after birth and skim milk to 6 months of age. A concentrate mixture was fed, which consisted of 30 per cent white corn or barley, 20 per cent wheat bran, 25 per cent soybean meal, and 25 per cent linseed meal, plus 2 per cent bonemeal and 1 per cent salt. This mixture contained about 0.5 microgram carotene per gram. Timothy hay low in carotene content was fed as soon as the calves would eat it. Sufficient nutrients were offered to enable the animals to make normal growth when adequate carotene or vitamin A was supplied. In no instance was more than 8.5 pounds of hay fed daily. In order to induce vitamin A deficiency in the bulls at various ages and yet prevent them from succumbing completely from this deficiency, the ration was supplemented with varied amounts of cod-liver oil for varied lengths of time. Cod-liver oil containing 600 micrograms of vitamin A per gram was used. In several instances the bulls were given carotene supplement either as alfalfa meal or as carotene in cottonseed oil after the appearance of marked symptoms of vitamin A deficiency, such as blindness, loss of appetite, diarrhea, and muscular incoordination

Each lot of hay was analyzed for carotene before it was fed as were also a number of samples of the concentrate mixture in the early phase of the experiment. The method of analysis was similar to that described in an earlier report from this laboratory (16) except that the carotene portion was filtered through an absorption column of magnesium carbonate.

The bulls were observed daily for the usual gross symptoms of vitamin A deficiency. The occurrence of total blindness, which was usually associated with fits and muscular incoordination, indicated advanced deficiency. At infrequent intervals the carotene and vitamin A content was determined for the blood plasma and for the livers of some of the bulls at autopsy. In the first part of the experiment the blood analyses were conducted by the method described by Davis and Madsen (1). Later a modification of this method was used in which the carotene and vitamin A were extracted from the plasma by the method of Davis and Madsen (1) after which the carotene was treated as described previously (16). The vitamin A determination was made by extracting the non-saponifiable matter with Skellysolve F, bringing to dryness and then dissolving in chloroform for analysis by the antimony trichloride color development at 620 millimicrons. Toward the end of the experiment a few samples of blood were analyzed for carotene and vitamin A by the method described by Moore (10). The carotene content of the liver was determined by the same method as was used with the feed (16); the vitamin A determination on liver was made from the alcohol extract which had been diluted with an equal volume of 10 per cent sodium chloride and then extracted by Skellysolve F. The latter extract was brought to dryness in vacuo and saponified. The non-saponifiable matter was extracted in Skellysolve F. brought to dryness in vacuo, and then dissolved in chloroform for analysis by the antimony trichloride color development at 620 millimicrons.

As the bulls reached 10 to 12 months of age they were given the opportunity to mount a cow and serve an artificial vagina. (In this paper, for sake of brevity, the term "breeding" is used to denote the act of mounting and ejaculating into an artificial vagina.) In some instances bulls showed interest but were too weak or uncertain of themselves to mount. With few exceptions attempts were made to collect semen from each bull once a week. Two ejaculates were collected when they were obtainable. In some cases collections were made more often while in others collections were not obtained weekly. If a bull showed no interest in the cow he was judged to be without normal breeding ability. When bulls would not serve the artificial vagina, semen frequently was obtained by massage according to the technique developed by Miller and Evans (9).

After the semen samples were collected they were brought to the laboratory without delay and were rated for motility, volume, pH, and sperm concentration according to the methods outlined by Lambert and McKenzie (6). Specimens of diluted semen were dried on glass slides, left unstained and examined for percentage of normal sperm—a total of 200 being counted for each production interval of 30 days for some bulls and 60 days in the case of others. Each sample of semen was stored undiluted at 4° C. and the motility after 48 hours was recorded. Semen from the bulls was used to test its fertilizing capacity as often as estrus cows were available.

As each bull finished the experiment an autopsy examination was made. Samples of various tissues were prepared and examined histologically.

RESULTS

The carotene content of the roughage and the amounts of vitamin A or carotene supplements and the ages at which the supplements were given are shown in table 1. Table 2 gives the length of time each bull was on the experiment, the age when deficiency symptoms first appeared, and the age when the first semen was obtained. The live weights of the bulls, compared with the normal growth curves for Holstein and Jersey bulls reported by Ragsdale (13), are shown in figure 1.

All except H-2106, H-126, and J-314 were decidely under-weight after the first 3 or 4 months. These three bulls began falling below the normal at from 10 to 12 months of age. The Red Danish x Jersey hybrid (X–132) cannot be compared with a normal but his growth curve also leveled off at about this age. Bulls H–2106, H–126, J–314, J–2324, and X–132 were more than 90 per cent of normal in weight at 10 and 13 months of age when they started to breed (table 2). These bulls received more vitamin A supplement or received it for a longer time than the others.

TABLE 1

Carotene content of the roughage and vitamin A or carotene supplement fed

Bull No.	Weighted average carotene content of roughage	Supplement fed at different ages (micrograms per day) * †						
		1	2	3	4	5	6	7
	mcg./g.							
H–2106	1.7	2–490 5426						
H–126	1.7	2–180 6000	575–637 8033‡	638–997 10986§				
H–130	1.7	32–80 1686	95–109 264	166–178 325	235–246 600	273–341 1020	373–503 591	504–694 600
H–245	1.9	2–111 1278	199–210 600	237–305 1108	337–467 600	468–646 300		
H–2174	2.2	2–130 1662	171–175 600	183–192 600	248–168 300			
H–256	1.8	2–114 1333	153–162 966	223–380 600				
J–314	1.6	2–90 7400	481–821 7176‖					
J–470	2.1	2–125 1301	424–540 300	577–653 3600‖	654–713 7200‖			
J-2311	2.3	2–125 1360	213–222 300	306–393 600	394–597 300	651–835 5400‖	836–936 10800‖	937–1065 75000‖
J-2320	2.2	2–133 1091	158–161 750	333–454 300				
J-2324	1.4	2–244 1312						
X–132	1.8	2–261 1190	543–905 8000‖					

* Unless otherwise indicated the supplement is vitamin A in cod-liver oil.

† In each column the top figure is the age in days when the bull was fed the supplement and the bottom figure is the amount fed.

‡ Carotene mixed in cottonseed oil and injected subcutaneously.

§ Carotene fed mixed in cottonseed oil.

‖ Carotene fed in the form of alfalfa meal.

The six animals that became blind before 300 days of age did not start to breed at the expected 10 to 12 months of age nor did J–470 even though he was not totally blind until 420 days old. The first semen was taken by massage from H–245, H–130, H–2174, J–470, J–2311, and J–2320 after it was apparent they would not breed. No semen was obtained from H–256 before his death at 380 days. They were all 77 per cent, or less, of the expected weight at the time the first sample was taken. These seven bulls received the least vitamin A supplement up to this time.

The bulls that started to breed at the expected age were not blind and two of them (H-2106 and J-2324) retained their breeding ability long after total blindness had occurred. Bulls H-126 and J-314 failed to breed shortly after going blind, possibly in part due to weakness and incoordination in the rear legs. Bull X-132 bred very irregularly soon after going blind and semen had to be taken from him by massage. These three animals regained their breeding ability following carotene supplementation although they remained blind.

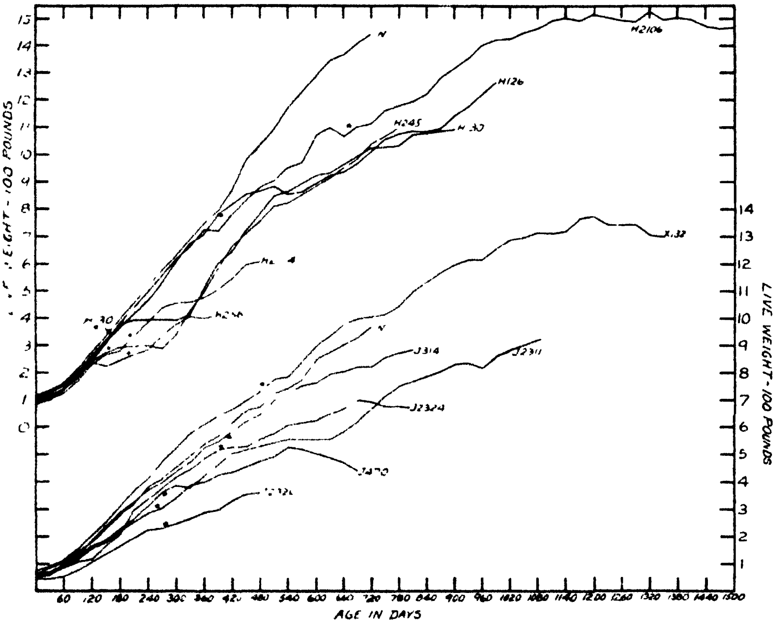

FIG. 1. Growth curves of bulls compared with respective breed average. *N* Normal growth curves for Holstein and Jersey bulls for first two years as published by Missouri station (13). X-132 is a Dane Jersey cross. Asterisk indicates the age at which total blindness occurred.

In addition to the small amount of carotene consumed in the hay (5.9 micrograms per pound weight) by H-126, daily subcutaneous injections of 8,033 micrograms of carotene (9.3 micrograms per pound weight) for 62 days followed by daily feedings of 10,986 micrograms of carotene enabled him to resume breeding 83 days after the first injection. J-314 was consuming about 6.0 micrograms of carotene per pound weight daily from the roughage and when 7,176 micrograms of carotene (11.1 micrograms per pound weight) was added to his daily ration he started to breed again within

16 days. Adding 8,000 micrograms of carotene (10.2 micrograms per pound weight) to the 6.9 micrograms carotene per pound of weight daily that X–132 was taking in the hay shortly after he had become a very irregular breeder caused him to start breeding almost immediately. After about a year the carotene supplement was stopped but he continued to breed even though he again became very weak.

Bull J–2311 did not breed until he was 651 days of age, which was 115 days after 5,400 micrograms of carotene (8.4 micrograms per pound weight) had been added to the 5.5 micrograms per pound weight that he was receiving in the hay. Semen had been previously obtained however by massage. H–245 was fed the same deficient basal ration as the other bulls but as shown in table 1 a small amount of vitamin A in addition, which was thought necessary for sustained life, was fed as cod-liver oil from 337 to 646 days of age. He gave semen only by massage from 433 days to 607 days of age after which he started to breed. From 433 days until the cod-liver oil was discontinued the amount of vitamin A fed averaged 348 micrograms daily or less than 0.5 microgram per pound weight. Bulls H–130, H–2174, J–470, and J-2320 failed entirely to breed. However a few samples of semen were obtained from each by massage. Apparently the small amount of vitamin A fed in addition to the carotene that these three bulls received in the basal ration (see table 1) after the first sample of semen was obtained by massage was not sufficient to enable them to breed.

TABLE 2

Ages of bulls at end of experiment, when total blindness occurred and when semen was first obtained and the per cent of normal weight when blindness occurred and semen was first collected

Bull No.	Age at end of experiment	Age when vitamin A deficiency appeared		Age when semen first obtained	Per cent of expected weight when	
		First fit	Totally blind		Totally blind	When first semen obtained
	days	*days*	*days*	*days*	%	%
H–2106	1517	676	685	306	78	100
H–126	997	430	396	317	99	101
H–130	911	151	152	456*	94	74
H–245	801	134	156	433*	80	77
H–2174	486	174	205	470*	74	68
H–256	380	225	222		51	
J–314	821		420	364	95	99
J–470	708	208	420†	505*	70	70
J–2311	1095	508	279	556*	90	76
J–2320	496	295	276	477*	56	54
J–2324	828	93	425‡	395	84	91
X–132	1347	450	481	363		

* Obtained by massage.
† Blind in one eye at 234 days of age.
‡ Blind in one eye at 281 days of age.

The blood plasma vitamin A and carotene of some of the animals determined at various times are given in table 3. The bulls that were maintained vitamin A deficient after becoming blind (H–2106, H–130, H–245, and J–2324) showed progressive decreases in the plasma vitamin A and carotene. Bulls H–126, J–314, J–470, J–2311, and X–132 supplemented with carotene after developing avitaminosis A showed increases in the plasma vitamin A and carotene content. Accompanying this increase was an increase in feed consumption, weight, and, except for J–470, in the ability to breed. The improvement in feed consumption and weight may have contributed much to the increased breeding ability. On the other hand, when carotene was added to the ration of J–470 the daily feed

TABLE 3

Vitamin A and carotene content of blood plasma and livers

Bull No.	Vitamin A and carotene content of blood plasma at different ages (micrograms per 100 cc. plasma) * †									Vitamin A and carotene in liver *
	1	2	3	4	5	6	7	8	9	
										mg./g.
H–2106	490 15 18	609 7 35	681 9 25	1106 8 23	1175‡ 7 15	1190‡ 6 12	1474§ 2 5	1495§ 3 5	1509§ 3 2	1517 0.004 0.005
H–126	479 5 11	506 4 11	590 6 18	605 6 19	637 15 29	665 11 46	725 12 53	796 18 56	997 16 63	
H–130	685‡ 6 14	720‡ 5 9	754‡ 2 7	911‡ 2						911 0.02 0.08
H–245	669‡ 3 6	712‡ 2 7	801‡ 1 7							801 0.08 0.12
J–314	402 8 14	462 6 10	537 8 33	592 13 44						
J–470	560‡ 5 10	601‡ 6 18	632‡ 6 32	708‡ 11 73						708 0.42 1.16
J–2311	635‡ 5 4	664‡ 7 15	682‡ 7 14	775‡ 8 15	804‡ 5 11	945§ 12 34	970§ 17 74	985§ 24 119		...
J–2324	481‡ 7 12	725‡ 3 8	748§ 2 6	767§ 2 7						
X–132	463 8 11	523 6 9	583 12 41	653 11 37	1078 13 22	1174‡ 3 8	1347‡ 5 4			1347 0.03 0.05

* In each column the top figure is the age of the bull, the middle figure the vitamin A content and the bottom figure the carotene content.

† Unless otherwise indicated the vitamin A and carotene was determined by Dr. L. L. Madsen according to the Davis and Madsen procedure (1).

‡ Vitamin A and carotene were determined by the procedure herein described.

§ Vitamin A and carotene were determined by Dr. L. A. Moore (10).

allowance was kept constant. His appetite become ravenous, he lost weight, and his ability to breed showed no improvement even though the plasma vitamin A and carotene increased. He remained in a very weak condition.

The bulls that were in advanced vitamin A deficiency when slaughtered had practically no vitamin A or carotene in their livers (table 3). The vitamin A and carotene content of the liver of J–470 was very low even though the carotene consumed in hay and carotene supplement was increased from 1,900 micrograms daily to 5,500 micrograms for 77 days, then

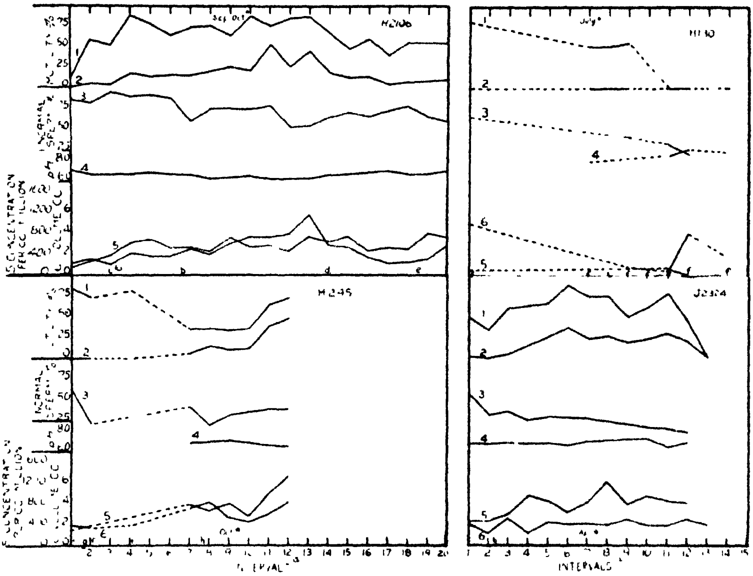

FIG. 2. Characteristics of semen. 1. Average per cent initial motility. 2. Average motility after storage. 3. Average per cent normal sperm. 4. Average pH. 5. Average number of sperm per cubic centimeter. 6. Average volume per ejaculate in cubic centimeters. *a*. Each interval represents the average for individual ejaculates obtained from the time semen was first obtained through successive 60-day periods for H–2106 and 30 day periods for the other bulls (dotted lines indicate that no samples were obtained during successive intervals). *b*. Total blindness occurred. *c*, *h* and *i*. Vitamin A supplement discontinued. *d*. Semen collections increased to three times weekly. *e*. 300 cubic centimeters mineral oil daily added to ration. *f*. Semen samples obtained by massage. *g*. Vitamin A supplement reduced from 600 to 300 micrograms daily. Asterisk indicates month or months during which samples representing this average were collected.

to 9,100 micrograms for an additional 60 days. This represented an increase of from 3.7 to 10.6, then to 20.0 micrograms per pound weight.

Figure 2 gives the pertinent data concerning the semen produced beginning with the first sample obtained either by the artificial vagina or by massage by four of the bulls (H–2106, J–2324, H–245, and H–130) that were not supplemented with carotene. Insufficient semen was obtained from

TABLE 4

Condition of anterior pituitary of bulls at autopsy

Bull No.	Description of pituitary
H-2106	Small cyst; AP about one-half size and not greatly damaged.
H-126	Large cyst; AP damaged but not extensively.
H-130	Very large cyst; AP little more than a shell, extensive pressure atrophy; probably not more than 10 per cent of normal tissue remaining.
H-245	” ” ” ” ” ” ” ” ” ” ” ”
H-2174	Medium sized cyst: AP showed little tissue damage.
H-256	Small cyst; AP showed small portion of tissue damaged.
J-314	” ” ” ” ” ” ” ” ”
J-2320	” ” ” ” ” ” ” ” ”
J-2324	” ” ” ” ” ” ” ” ”
J-470	Large cyst; AP approximately 65 per cent destroyed.
X-132	Large cyst; AP little more than a shell, probably only 20 per cent of normal tissue remaining.

H-2174, J-2320, and H-256 for graphic analysis. Table 4 gives a description of the condition of the pituitaries and figure 3 shows photomicrographs of the condition of the testes of these bulls when slaughtered. Table 5 gives the fertility records.

TABLE 5

Fertility of the bulls

Intervals *	First figure is the number of times semen was used and second figure the number of conceptions during each 30-day interval								
	H-2106†	X-132†	H-126†	H-130	H-245	J-314	J-470	J-2311	J-2324
1	0	0	0	0	2-0	0	0	0	5-2
2	0	0‡	0‡	0	3-1	0‡	4-1	3-1	0‡
3	1-1	1-0	1-0	0	0	0	3-1§	2-0	2-1
4	0	1-0	2-2	0	2-2	0	0	0	0
5	3-2	0§	0§	0	0	1-1§	0	0	0
6	4-2	0	0	0	0	0	0	0	1-0
7	2-2	1-1	1-1	0	0	0	0	1-0	0
8	0	0	1-0	0	1-0	0		0	0
9	1-0	0	2-0	2-0	1-0	0		1-0	0
10	2-2	4-0‖	2-0	0	2-1	0		1-1§	0
11	1-1	6-3	4-3	0	1-1	0		1-0	0
12	1-1	0	0	0	1-1	0		0	0
13	0	0		0		0		0	
14	4-2	0		0		0		0	
15	6-2	0				0		0	
16	2-0	1-0						0	
17	1-0							1-1	
18	3-1								
19	3-1								
20	4-2								

* Intervals conform with semen production periods shown in figures 2 and 4.

† Sixty-day intervals.

‡ Bull went blind in this period.

§ Supplemental carotene started in this period.

‖ Supplemental carotene was discontinued at end of this period.

The average motility of the freshly collected semen from H–2106 increased steadily during the first eight months (four 60-day intervals) but as the bull developed vitamin A deficiency (685 days) this trend did not continue. The motility after storage followed the same general trend but at no time was the keeping quality good. The percentage of normal sperm was above 80 before blindness occurred but thereafter it was usually below this figure and decreased to less than 50 per cent during some intervals. The pH of the semen varied between 6.3 and 7.2 with a gradual tendency toward alkalinity after the number of collections per week was increased. The number of sperm per cubic centimeter increased gradually during the first five intervals (306 to 606 days) and then fluctuated between 400 and 800 million per cubic centimeter showing no definite trend. At 1,095 days of age semen collections were increased from two to three times per week with two ejaculates being taken each time in an unsuccessful effort to exhaust his semen production. The volume of semen gradually rose through the first 13 intervals but after the number of collections per week was increased the volume and motility decreased. When he was 1,380 days old and until slaughtered, 300 grams of mineral oil was fed daily to reduce absorption of the small amount of carotene in the feed. In addition five grams of yeast daily was fed as a source of vitamin D. This bull had a small pituitary cyst (table 4) and the germinal epithelium was markedly degenerated (fig. 3, *a*) when he was slaughtered at a little over four years of age. The semen was used just before blindness occurred and throughout the following long period when he was becoming progressively more deficient (table 5). In all, 38 cows were inseminated and 19 of them conceived, the last one within 30 days before the bull was killed.

Semen was collected from J–2324 for about 13 months, beginning about 30 days before he became blind. His semen showed an irregular increase in initial and storage motility, concentration, and pH over the first eleven 30-day intervals (figure 2). During the last two intervals the initial and storage motility dropped sharply. He was very weak from vitamin A deficiency during this time. The volume averaged below 2.5 cubic centimeters throughout. The percentage of normal sperm decreased steadily and was very low at all times. This bull had a medium sized pituitary cyst that had produced some damage to the anterior lobe tissue (table 4) and there was marked degeneration in the tubules of the testes (fig. 3, *d*). His semen was used to inseminate five cows in the month preceding blindness and two of them conceived. It was used three times after blindness occurred and one of the cows become pregnant.

The semen from H–245, obtained by massage, was very low in concentration and contained a high percentage of abnormal sperm. The motility of these samples was high but this was not maintained after storage. The semen collected after he started breeding improved steadily in initial

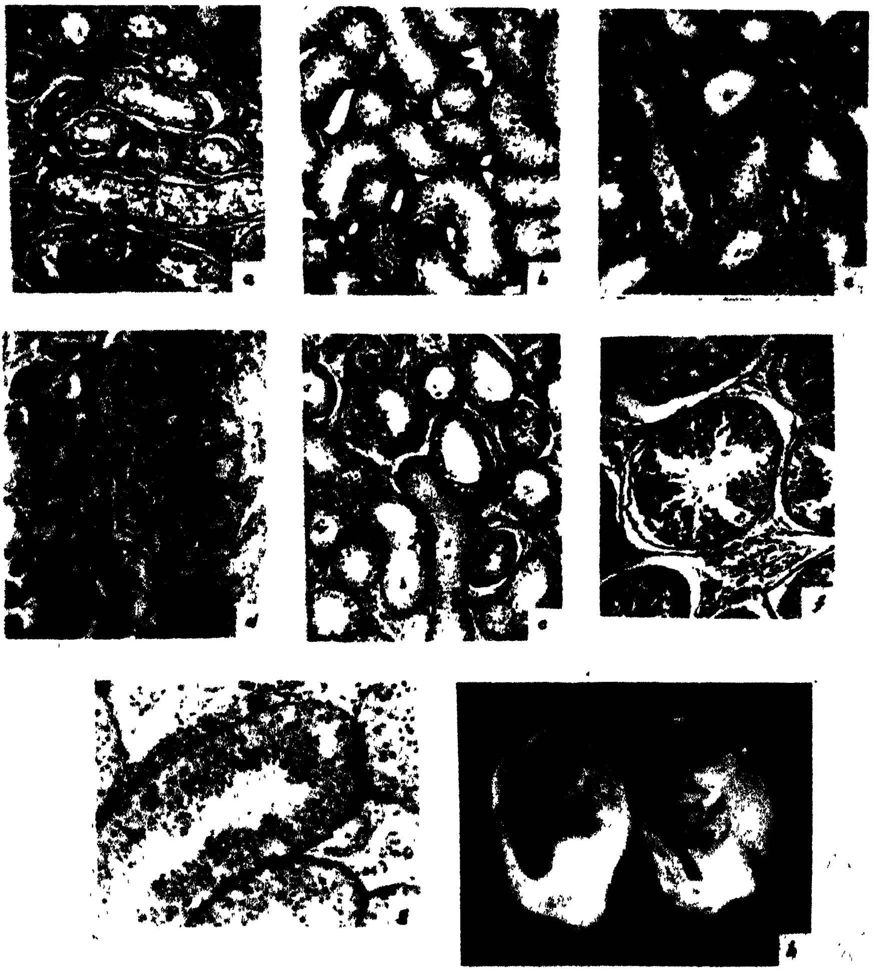

Fig. 3. Photomicrographs of section of testes cut at 7–8 micra and stained with Delafield's Haematoxylin. *a.* H–2106 at autopsy. This animal, sacrificed at a little more than four years of age, was proved fertile about a month before this specimen was obtained. The final stages of depletion were hastened by the addition of mineral oil to his ration. × 75. *b.* H–130 at autopsy. He also received mineral oil during the final months. His anterior pituitary was severely damaged (see *h* below). × 75. *c.* H–245 at autopsy. His pituitary was damaged as severely as H–130 and he had marked vitamin deficiency symptoms. H-245, however, was not given mineral oil toward the end of the experiment. Note the remarkable preservation of the germinal epithelium. × 75. *d.* J–2324 at autopsy. Note the decrease in the diameter of the tubules. × 75. *e.* J–2320 at autopsy. An occasional sperm could be found in the lumen of the tubules. × 75. *f.* H–2174 at autopsy. Early stages in degeneration of the germinal epithelium. × 210. *g.* H–256 at autopsy. Note the vacuolation in the otherwise fairly normal germinal epithelium. × 210. *h.* H–130. Little more than a "shell" remains of the pituitary gland as a result of the formation of the cyst. At the lower end of the photograph is the posterior lobe which was not as damaged as the anterior.

and storage motility, concentration, and percentage of normal sperm. The pituitary weighted 18.5 grams and contained a large cyst. The germinal tissue however contained a high percentage of apparently normal cells and numerous sperm could be seen in the lumen (fig. 3, *c*). As shown in table 5, semen obtained by massage was used to inseminate seven cows and three of them became pregnant. Later when the bull was serving the artificial vagina his semen was used six times and three cows were settled.

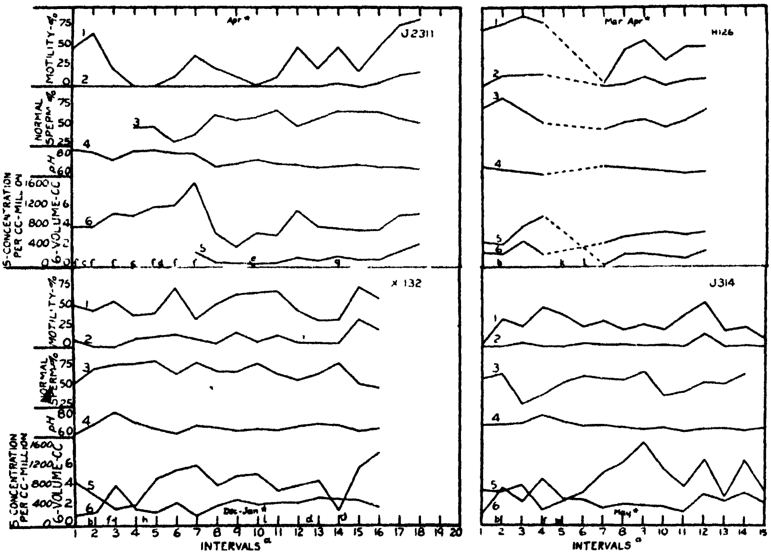

FIG. 4. Characteristics of semen. 1. Average per cent initial motility. 2. Average motility after storage. 3. Average per cent normal sperm. 4. Average pH. 5. Average number of sperm per cubic centimeter. 6. Average volume per ejaculate in cubic centimeters. *a*. Each interval represents the average for individual ejaculates obtained from the time semen was first obtained through successive 60-day periods for bulls H–126 and X–132 and for 30-day periods for bulls J–314 and J–2311 (dotted lines indicate that no samples were obtained during successive intervals). *b*. Total blindness occurred. *c*. Vitamin A supplement discontinued. *d*, *h*, *l*, and *m*. Supplemental carotene added to daily ration. *e* and *g*. Carotene supplement increased. *f*. Semen samples obtained by massage. *j*. Carotene supplement discontinued. *k*. Carotene supplement injected subcutaneously. Asterisk indicates month or months during which samples representing this average were collected.

Although H–130 received a little more cod-liver oil and for a little longer time than did H–245 after going blind, semen was obtained from him only by massage. It contained a small number of sperm, only a few of which were motile (fig. 2). The pituitary of this bull was damaged extensively (table 4 and fig. 3, *h*) as was the germinal epithelium (fig. 3, *b*). His semen was used twice in the ninth interval but neither time successfully.

Only a few samples of semen were obtained from H–2174 and J–2320 and these were by massage. Neither animal was tested for fertility. Both had pituitary cysts which had damaged the anterior lobe tissue (table 4) and they had considerable degeneration of the germinal epithelium (fig. 3, *f* and *e*, respectively).

Bull H–256 was considerably undersized for his age and semen was never obtained from him by either method before his death from pneumonia at 380 days. He had a small pituitary cyst at the time of death (table 4) but the germinal layer (fig. 3, *g*) shows only a small amount of degeneration and a few sperm were apparent in the lumen.

Bulls H-126, X–132, J–314, J–2311 and J–470 make up the group that received supplemental carotene after blindness had developed (see figs. 4 and 5, and tables 4 and 5).

The semen collected from H–126 during the time he was developing vitamin A deficiency gradually improved in motility and concentration while the per cent normal sperm and pH decreased. The volume of semen averaged only 1.5 to 2.5 cubic centimeters. The semen produced after supplemental carotene was added in general increased in motility, percentage of normal sperm, and volume. However, the initial and storage motility did not improve and the concentration remained very low. The pH gradually changed from about 7.1 to 6.4. Autopsy examination revealed a large pituitary cyst (table 4). A biopsy specimen of the testes, taken shortly after blindness developed and during the time when he would not breed, showed some germinal tissue degeneration (fig. 5, *b*); while after carotene feeding, as shown in figure 5, *c*, this tissue was improved but not entirely recovered. His semen was used to inseminate three cows between the time he went blind and the time he no longer would breed and two of them were settled (table 5). Ten cows were inseminated after he had resumed breeding following carotene supplementation and four of them became pregnant.

The semen obtained from X–132 both by breeding and by massage before carotene supplementation contained sperm which were poor in motility, low in concentration, and high in pH (fig. 4). After carotene was added and the bull resumed breeding the concentration increased (but not to a normal level) and the initial and storage motility improved slightly. There was little change in the volume, percentage of normal sperm, and pH. When the carotene supplement was discontinued the initial motility decreased, the pH increased, and the storage motility and volume showed no change. Increasing the collections from two to three times per week produced a slight decrease in concentration and an increase in percentage of normal sperm. When 15,000 units of pregnant mare serum were given subcutaneously during a period of 24 days, beginning with the fourteenth interval, a sharp rise occurred in the initial and storage motility, concentration, and percentage of normal sperm. The increased motility did

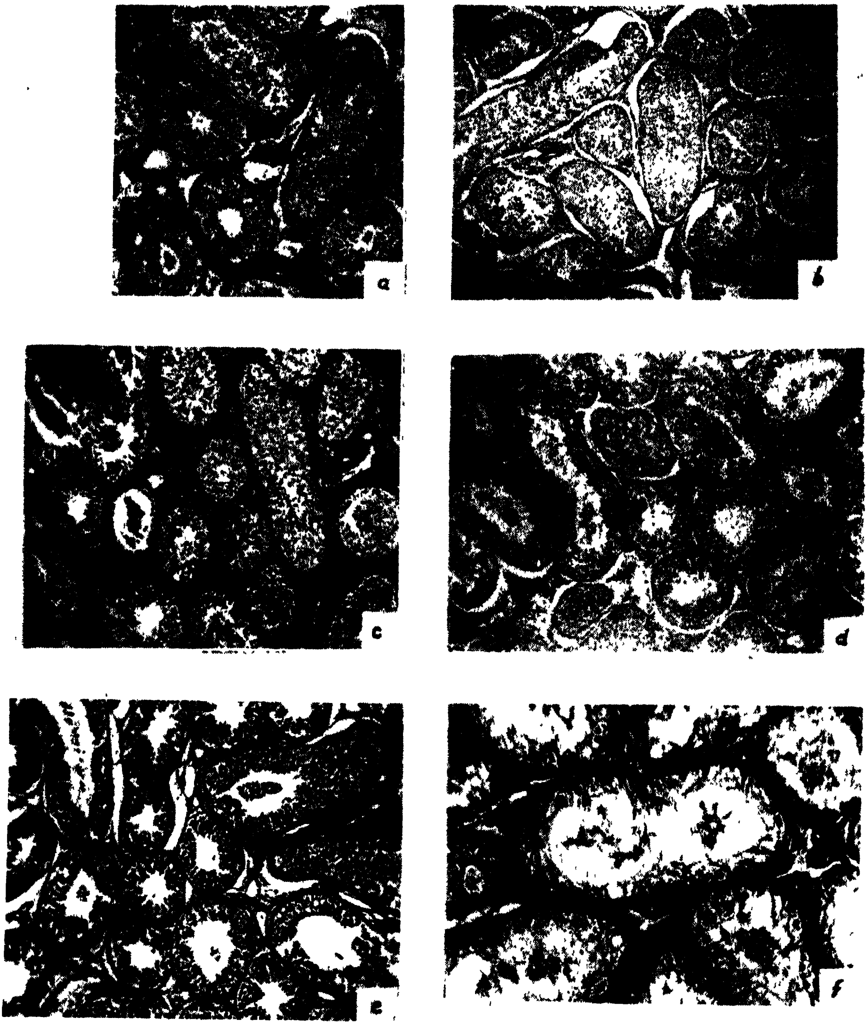

FIG. 5. Photomicrographs of section of testes cut at 7–8 micra and stained with Delafield's Haematoxylin. *a*. Normal mature bull. × 75. *b*. Biopsy of H–126 shortly after blindness occurred on a vitamin A deficient ration. At this time he was too weak to breed and no massage samples of semen were collected. From the appearance of the testes at this time he was probably producing many normal sperm. × 75. *c*. H–126 at autopsy. The general, although not complete improvement following limited carotene therapy may be seen. × 75. *d*. J–314 at autopsy. This animal also was sacrificed after a period of carotene therapy. Notice the normal appearance of much of the germinal epithelium. × 75. *e*. X–132 at autopsy. This animal was sacrificed after a second depletion period. Note the fairly normal condition of the germinal epithelium and sperm are present in the lumen of the tubules. × 75. *f*. J–470 at autopsy. This animal received limited carotene therapy but his energy intake was held down. Note the complete absence of spermatogenesis. × 210.

not persist long after the treatment was stopped but the concentration remained high to the end of the experiment. This bull had a pituitary cyst (table 4) and the germinal tissue showed some degeneration (fig. 5, *e*). The semen was used during his first depletion to inseminate two cows but without success. Later, during the carotene-feeding period and the second depletion, his semen was used to inseminate 12 cows and four became pregnant.

The semen produced by J-314 (fig. 4) throughout the experiment was of very poor initial motility and had practically no motility after storage. During no interval were the sperm more than 70 per cent normal. The pH of the semen was more than 7.0 during the first seven intervals and between 6.5 and 7.0 thereafter. The concentration of sperm varied between 3.5 and 4 hundred million per cubic centimeter until supplemental carotene was given, after which it increased to over 16 hundred-million in the ninth interval, then ranged between 6 and 12 hundred million. This bull had only a small pituitary cyst (table 4) and the germinal epithelium showed some irregular areas of damaged tissue (fig. 5, *d*). The semen was used only once in the fifth interval, 24 days after carotene was added to the ration, and the cow became pregnant.

The sperm obtained from J–2311 by massage varied in initial motility from sample to sample and they did not keep well in storage. Although the concentration was not determined it was extremely low and less than 50 per cent of the sperm appeared normal. During this time his fertility was tested with six estrus cows, only one of which conceived. The semen produced after he started to breed following carotene supplementation was very low in concentration and the motility varied considerably. It did not store well and the pH ranged between 7.0 and 7.2. There was a gradual increase in the percentage of normal sperm as the amount of carotene fed increased (table 1). Six cows were inseminated with his semen after he started to serve the vagina and three of them settled.

While J–470 yielded semen by massage occasionally, not enough was obtained for graphic analysis. When he was slaughtered a pituitary cyst of medium size was found and, as shown in figure 5, *f*, marked degeneration had occurred in the germinal epithelium which carotene therapy apparently had not corrected in the absence of an adequate feed intake. Seven cows were inseminated with his semen while he was in advanced vitamin A deficiency and shortly before carotene was fed. Of these seven, two became pregnant.

Microscopic examination of preparations obtained at autopsy of several organs and tissues from the bulls including the adrenals, thyroid and kidneys, muscle and small intestines, showed no pathological changes. Scrapings of the epithelium of the trachea obtained at autopsy of the bulls in the most advanced stages of vitamin A deficiency (H–2106, H–130, H–245 and

J-2324) showed active ciliated cells in all cases. Changes in the bony structure of the head including constriction of the optic foramen was noted in the bulls, and particularly in H-130 and H-245 where the bony pocket encasing the pituitary body was much enlarged, probably caused by pressure from the cystic gland. The skull of H-130 particularly was spongy and the bone brittle.

DISCUSSION

One important effect of a marked vitamin A deficiency on the reproduction function of dairy bulls is the inability to mount and deliver semen. The extent to which this function is impaired or retarded depends somewhat on the age at which deficiency occurs and the severity of the deficiency. The bulls that were given enough vitamin A to make satisfactory growth up to the age when they were expected to start breeding in most instances did so and continued to breed for long periods even though marked deficiency symptoms appeared. Those developing avitaminosis A at about the time they started to breed, frequently failed to breed shortly after going blind. However, they bred following the addition of small amounts of supplemental carotene to the basal ration (8 to 11 micrograms per pound daily bringing the total daily consumption to between 14 and 20 micrograms per pound). This confirms the work of Hart (4) and Madsen *et al.* (12). The bulls that showed marked deficiency before they were 8 or 9 months of age did not start to breed at the expected age, although when supplemental carotene was added at older ages dramatic improvement occurred. The fact that the bulls could not see apparently was not the cause of their lack of desire or ability to breed, since three of the blind bulls continued to breed for a long time while remaining on the deficient ration and four others also bred when the ration was supplemented with carotene even though they remained blind.

The occurrence of a pituitary cyst with its accompanying damage to the anterior lobe may be the cause of failure in the development of breeding ability at the expected age because of a reduced secretion of pituitary hormones. Bulls that had the largest cysts or the greatest damage to the anterior lobe generally had been the poorest breeders and the most retarded. However H-245 having the largest cyst, had started to breed at a late age but bred regularly after he started. Also, the bulls that had pituitary cysts and that had not bred developed this function when fed supplemental carotene, although the cyst persisted. Sutton *et al.* (14) also observed gross pituitary damage and some evidence of histological changes in this gland. Inanition and impaired growth accompanying advanced vitamin A deficiency may be at least partly responsible for breeding failure. Weakness, incoordination, and a feeling of uncertainty in mounting may also be contributing factors to this failure. When J-470 was given carotene without increasing his feed intake there was no improvement in breeding ability.

The seminal fluids obtained by either method from vitamin A deficient bulls invariably contained sperm. In most instances the sperm were motile. The sperm did not maintain motility after storage, were low in concentration, and a high percentage of them were abnormal in shape. The pH of the semen tended to increase in very advanced stages of vitamin A deficiency. The semen, although of apparently poor quality, was frequently successfully used to settle cows. Bulls were proved fertile while in advanced deficiency when showing blindness, incoordination, weakness, diarrhea, and edematous joints, low blood plasma carotene and vitamin A and, at autopsy, cystic pituitaries and degenerated testicles. Calves born to normally fed cows bred to these vitamin A deficient bulls delivered calves that were normal in every respect.

It is apparent from these results that gross vitamin A deficiency symptoms such as impaired eyesight and incoordination, etc., are apt to appear before any serious impairment in a bull's reproductive performance occurs. Under practical feeding conditions these symptoms are not often encountered. The quality of the roughage used in these experiments to produce vitamin A deficiency was poorer than that usually available on most farms.

Even though a number of the vitamin A deficient bulls in these experiments were fertile their semen was low in concentration, contained a high percentage of abnormal sperm, and lacked staying qualities—factors that may limit the bulls' usefulness, particularly in artificial insemination associations. The minimum level of vitamin A feeding that will permit bulls to produce high quality semen over long periods of time remains to be determined.

SUMMARY

Typical deficiency symptoms (total blindness, incoordination, emaciation, and extremely low blood plasma carotene and vitamin A values) were made to appear in 12 dairy bulls at different ages by adjusting the vitamin A intake.

Seven of the animals (all that were tested more than twice) were proved fertile by artificially inseminated cows. The semen used was usually obtained in an artificial vagina but it had to be collected by massage from the animals that would not mount. These bulls were also fertile.

Generally the semen was low in concentration, had a high percentage of abnormal sperm, a high pH, and did not store well.

When gross vitamin A deficiency symptoms appeared before the expected breeding age, the bulls failed to breed; when symptoms appeared at about the expected breeding age, the bulls frequently failed to breed

shortly thereafter; but when the deficiency became apparent after they had started breeding, this capacity was maintained.

At autopsy all the bulls were found to have cystic pituitary glands. The degree of damage to the anterior lobe varied, probably less than one-fifth normal tissue remaining in four bulls,—three of which incidentally were among those proved fertile. The animals exhibiting avitaminosis A earliest in life generally showed the greatest damage to this gland.

The epithelium of the seminiferous tubules was found to have undergone degeneration—some extensively. This epithelium, the anterior lobe of the pituitary gland, and the bones of the skull were the only tissues of several examined that had become altered.

Four of the bulls were fed rations supplemented with small amounts of carotene (8 to 11 micrograms per pound) after the appearance of avitaminosis A. This caused them to start breeding but the quality of the semen generally was not substantially improved. The testes of these animals were generally in better condition at autopsy than those of animals that did not get supplemented rations—giving evidence that carotene had brought about repair.

The germinal epithelium of one vitamin A deficient bull whose ration was supplemented but whose feed consumption was restricted was badly damaged, indicating failure to regenerate.

It is likely that gross vitamin A deficiency symptoms will appear before severe impairment to a bull's reproductive performance will occur.

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METHODS OF TESTING 80 PER CENT CREAM FOR BUTTERFAT

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In recent years, the process of manufacturing cream containing about 80 per cent butterfat has become commercially practical. Since no work has been published relative to the fat testing of this product, the following report is presented to suggest a procedure for purposes of control and billing "80 per cent cream."

Conditions which complicate the determination of butterfat in 80 per cent cream are: (a) obtaining a representative sample, (b) large amount of butterfat present, (c) rapidity of evaporation of moisture. (d) degree of accuracy required, and (e) speed of analysis required for control purposes.

Since the processes now in use for the production of 80 per cent cream are largely continuous, it is obvious that condition (a) above depends largely upon the uniformity of the separation process. If there is a wide variation in the fat test of the 80 per cent cream in a given lot, more samples are required to make a composite which is representative of that lot.

The buyer also must obtain a composite sample which is representative. His sampling may be complicated by the fact that the product is frozen and by his inability to sample containers at uniformly distributed intervals throughout the lot.

The large amount of butterfat in the cream presents the same difficulties encountered in testing butter. In extraction procedures, for example, it is necessary to use great care to insure complete extraction of the fat. In weighing the sample it is necessary to weigh more accurately than for a product containing a small amount of butterfat.

In order to mix properly a sample of 80 per cent cream, the temperature is such that moisture evaporates quickly. Samples held in partly filled jars or in loosely covered jars may lose as much as one per cent moisture in four hours.

Since 80 per cent cream is sold on the basis of fat content and since large amounts of butterfat are represented by a single test, it is apparent that the test should be within 0.2 per cent of the true amount of butterfat represented. This requirement automatically eliminates the Babcock test from consideration.

For control purposes the most desirable test is one which will yield results of the desired degree of accuracy in less than ten minutes. This condition eliminates the possibility of the Mojonnier test to be used as a

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control test, even though it was considered to be the test likely to be most accurate.

MOJONNIER METHOD FOR 80 PER CENT CREAM

The results of Herreid *et al.* (2) show that the efficiency of recovery of pure dry butteroil by the Mojonnier method using two extractions on 32 samples ranged from 98.4833 to 100.2123 per cent with an average of 99.5143 per cent. Assuming a fat content of a cream sample to be 80.00 per cent, these results would indicate a recovery of from 78.79 to 80.17 per cent with an average of 79.611 per cent. In view of this a similar study was made of the Mojonnier method using pure dry butteroil. The results of this study showed that in order to obtain an average of 100.00 per cent recovery, it was necessary to make the following modifications:

The Mojonnier extraction flask was suspended from the stirrup of the analytical balance, weighed, and a one-gram sample of 80 per cent cream injected into the flask with a 2-cc. syringe (without needle) avoiding contact of the syringe with the flask. The flask was quickly weighed again, the increase in weight being the weight of the sample. Water, ammonia, and alcohol were added and the contents mixed without stoppering the flasks. This procedure greatly reduced the hazard of blowing out fat. The extraction time was increased to one minute of vigorous shaking. Neoprene stoppers were used. Thirty-second centrifugings were found sufficient. Three extractions were found to be necessary instead of two as in the standard Mojonnier method. Blank determinations were made on each batch of reagents used. All determinations were made in duplicate and those not agreeing by 0.1 per cent were re-run. All Mojonnier tests reported (except where noted in tables 1 and 2) were run according to the above technic.

KOHMAN METHOD FOR CHILLED 80 PER CENT CREAM

Overman and Okimoto (4) compared the Kohman method with the Mojonnier method on eight samples of butter. Their results averaged 81.851 per cent fat for the Mojonnier and 81.825 per cent for the Kohman method.

The method followed in this report is the same as approved by the committee of the American Dairy Science Association (1). When this method was applied to chilled 80 per cent cream it was noticed that the curd (milk-solids-not-fat) averaged slightly more than 10 per cent of the moisture. Because the Kohman method for fat in 80 per cent cream took more than twenty minutes, it was decided to study a method of arriving at the fat content by determining the moisture content of the 80 per cent cream.

KOHMAN CALCULATED METHOD FOR CHILLED 80 PER CENT CREAM

In early experiments, the Kohman method (1) as used for butter moisture was followed (except that the cup was cooled in water and carefully dried before weighing) and ten per cent of the moisture was added to the moisture and this sum subtracted from 100 to obtain the per cent fat. For example, if the Kohman moisture were 16.5 per cent, 10 per cent of this is 1.65 per cent. One hundred per cent minus the sum of 16.5 per cent and 1.65 per cent (or 18.15 per cent) is 81.85 per cent butterfat.

Later work (5) showed that more accurate results could be obtained by determining the exact percentage of the moisture which the curd represented. This percentage of the moisture was called the "factor" and was obtained as follows:

$$\text{Factor} = \frac{100 - (\%\ \text{Kohman moisture} + \%\ \text{Mojonnier fat})}{\%\ \text{Kohman moisture}}$$

In practice, this factor was determined weekly on a composite sample on one lot. Since slight variations were found, tables were prepared so that with a given factor the Kohman moisture could be read directly as per cent butterfat.

The practice at this plant has been to consider 500 packages of 80 per cent cream as one lot. As these packages were filled every tenth package was sampled and tested by the Kohman Calculated Method for fat. After 500 packages were filled the lot was completed and the 50 tests run during the lot were averaged. This average test was used for billing purposes. Once a week a composite sample was made up from every twentieth can. This composite sample was tempered at 100–110° F., well mixed with a malted milk mixer, and tested for fat by the Mojonnier method using three extractions. All necessary precautions were observed during the sampling and mixing operations to reduce to a minimum the loss of moisture from the sample. The Kohman moisture was also run

TABLE 1

Recovery of pure fat by the Mojonnier method

	Wt. sample	% Recovery
Using two extractions	0.5194	99.96
	0.6866	100.01
	0.7958	99.89
	0.9367	99.82
	1.4389	99.86
	1.5949	99.80
	1.6780	99.83
	1.7130	99.66
	1.7630	99.62
	1.8429	99.58
	2.2948	99.57
Using three extractions	0.9835	100.05
	1.2167	99.98
	1.9749	100.00
	2.0036	99.94

TABLE 2

*Comparison of standard Kohman and standard Mojonnier methods when applied to 80 per cent cream**

Sample No.	Kohman			Mojonnier Fat
	Moisture	Curd	Fat	
1	18.60	1.90	79.50	79.34
2	19.38	2.15	78.47	78.13
3	17.75	1.70	80.55	80.72
4	16.68	1.60	81.72	81.45
5	15.78	1.60	82.62	82.63
6	18.38	2.17	79.45	79.63
7	18.20	1.80	80.00	80.10
8	17.33	1.77	80.90	80.74
9	17.20	1.95	80.85	81.09
10	17.73	1.97	80.30	80.33
Average			80.438	80.416

* All tests run in duplicate and averaged.

on this same sample and the factor to be used for the following week was calculated using the formula given above.

TABLE 3

*Variations in "factor" as determined on various composite samples of extra heavy sweet cream**

Sample No.	Mojonnier fat	Kohman moisture	Calculated factor
1	79.66	18.45	0.1024
2	78.94	19.05	0.1050
3	79.46	18.60	0.1044
4	78.70	19.25	0.1049
5	79.65	18.40	0.1059
6	80.24	17.80	0.1101
7	80.40	17.80	0.1010
8	79.72	18.40	0.1022
9	79.92	18.20	0.1033
10	80.25	17.90	0.1028
11	79.30	18.80	0.1012
12	79.02	18.90	0.1101
13	80.12	17.90	0.1101
14	79.03	19.00	0.1037
15	79.79	18.40	0.0984
16	81.70	16.60	0.1063
17	81.00	17.30	0.0983
18	80.30	17.85	0.1036
19	79.75	18.55	0.0916
20	81.01	17.30	0.0976
21	79.64	18.50	0.1005
22	80.58	17.55	0.1065
23	79.69	18.60	0.0919
24	80.15	18.10	0.0967
25	79.45	18.70	0.0989
26	79.41	18.55	0.1100
27	80.49	17.85	0.0929
28	80.52	17.70	0.1006
29	80.31	17.90	0.1000
30	81.08	17.15	0.1032
Average	. .	.	0.10214

* All determinations run in duplicate and averaged.

TABLE 4

*Comparison of Mojonnier fat test of composite sample and average of calculated Kohman fat of 50 individual samples making up composite sample**

Sample Number	1 Composite (Moj. Fat Test)	2 Ave. of 50 tests by Kohman Calc. Method	3 Difference (Col. 1 – Col. 2)
1	80.68	80.48	0.20
2	79.36	79.32	0.04
3	80.29	80.52	– 0.23
4	80.75	80.77	– 0.01
5	81.24	81.22	0.02
6	80.20	80.33	– 0.13
7	80.11	79.94	0.17
8	80.95	80.91	0.04
9	80.39	80.23	0.16
10	80.58	80.70	– 0.12
11	79.83	79.76	0.05
12	79.38	79.32	0.06
13	81.02	80.95	0.07
14	80.38	80.64	– 0.26
15	80.60	80.79	– 0.19
16	80.78	80.83	– 0.06
17	80.73	80.57	0.16
18	80.68	80.76	– 0.10
19	80.97	81.08	– 0.13
20	81.34	81.15	0.19
21	81.55	81.33	0.22
22	81.19	81.00	0.19
23	81.10	81.10	0.00
24	80.70	80.63	0.07
25	80.94	81.02	– 0.08
26	80.48	80.40	0.08
27	79.92	79.90	0.02
28	80.45	80.52	– 0.07
29	81.10	81.25	– 0.15
30	80.50	80.52	– 0.02
31	80.65	80.37	0.28
32	80.10	80.26	– 0.16
33	80.07	80.08	– 0.01
34	79.79	79.98	– 0.19
35	81.19	80.95	0.21
36	81.10	80.93	0.17
37	82.07	81.79	0.28
38	82.00	82.09	– 0.09
39	80.50	80.75	– 0.25
40	82.42	82.29	0.13
41	80.80	80.61	0.19
42	80.74	80.58	0.16
43	81.46	81.69	– 0.23
44	81.38	81.13	0.25
45	81.08	81.12	– 0.04
46	80.30	80.27	0.03
47	80.48	80.25	0.23
48	80.38	80.16	0.22
49	79.41	79.39	0.03
50	80.15	80.18	– 0.03
Average	80.6854	80.6562	0.0292

* All determinations run in duplicate and averaged.

RESULTS

Table 1 gives the percentage recovery of pure dry butterfat when two extractions are used and when three extractions are used. Good recovery was obtained when less than 1 gram of butter was used but the per cent recovery dropped as the weight of the sample was increased when only two extractions were made. Best results were obtained when one or two grams of butterfat were extracted three times.

The accuracy of the Kohman method for 80 per cent cream is given in table 2. The fat test of 10 samples averaged 80.438 per cent by the Kohman and 80.416 per cent by the standard Mojonnier method.

Table 3 gives the variations in the factor that may be expected over a period of a year. The factor ranged from 0.0916 to 0.1101 with an average of 0.10214. On the basis of 18.3 per cent moisture in a given sample of 80 per cent cream, this variation would yield a Kohman calculated fat test of from 79.72 to 80.02 per cent. This range of 0.3 per cent represents the degree of accuracy that may be expected by testing an individual sample of 80 per cent cream for fat by the Kohman calculated method.

Table 4 gives a comparison of the Mojonnier fat test on a composite sample of a lot with the average Kohman calculated fat test of fifty samples of cream from the same lot. The Mojonnier test of the composite averaged 80.6854 per cent fat, the average of 50 tests made by the Kohman calculated method on the same lots was 80.6562 per cent, and the Mojonnier was from 0.26 per cent lower to 0.28 per cent higher than the Kohman calculated method.

CONCLUSIONS

The Mojonnier Method using three extractions was considered to be the most accurate method for testing 80 per cent cream.

On ten samples of 80 per cent cream, the Kohman Method averaged 80.438 per cent fat and the Mojonnier Method averaged 80.416 per cent fat.

A rapid control test, suitable for billing purposes, was described as the Kohman Calculated Method for chilled 80 per cent cream.

The average test of 50 lots of 80 per cent cream tested by the Kohman Calculated Method was 80.6562 per cent and the average test of a composite sample of each lot run by the Mojonnier Method was 80.6854 per cent.

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FERTILITY OF BULL SEMEN DILUTED AT 1:100

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The studies to determine the optimum rate of dilution of bull semen, which have been underway for several years in our laboratory (1, 2), have been continued. The earlier reports dealt with dilutions, in the first case of as high as 1 part of semen to 16 parts of the yolk-citrate diluent (1), and, in the second case, with dilution rates as high as 1:50 (2). No statistically significant differences in fertility among the dilution rates were found in either experiment. The results of the two experiments differed in total over-all fertility level. However, this was attributed not to the dilution rates used but to differences in the fertility of the bulls available for study.

In the present experiment the dilution potential has been pushed farther towards the supposed inevitable point were reduced efficiency of conception might be expected (2). However, in this as in the previous studies no obvious effect of the lower number of spermatozoa used in insemination has manifested itself. While these investigations are part of a plan to attempt to establish the minimum number of bull spermatozoa required for efficient fertility, the practical importance of these investigations for artificial breeding units is obvious. Therefore, the results, though not answering the fundamental problem, are being presented here for use by others.

EXPERIMENTAL

The experiment was conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc. A total of 19 bulls were made available, 11 Holstein-Friesians and 8 Guernseys. The precautions and methods used in the earlier investigations (1, 2) have been continued. However, as the earlier results had indicated that the precautions taken to standardize the use of all treatments rigorously within each collection period were not necessary the Latin square design previously used was abandoned. In its place a randomized block design was used. Each bull was considered as a block, and for each of 4 semen collections, spaced about 10 days apart, dilution rates were assigned at random. The dilution rates used were 1:40, 1:60, 1:80, and 1 part of semen to 100 parts of yolk-citrate diluent.

The effectiveness of this method for assuring uniform semen for the various dilutions is indicated from the average data for each dilution rate given in table 1. A statistical analysis of the semen characteristics showed highly significant differences between bulls, but no significant differences between dilution rates for the percentage of motile spermatozoa on initial examination, for concentration of spermatozoa per mm.^{3}, or for methylene blue reduction time.

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TABLE 1

Average data for semen used at each dilution rate

	Ratio of semen to diluter			
	1:40	1:60	1:80	1:100
Initial motility, %	78.4	79.4	79.4	79.4
Concentration, 1000's/mm.^{3}	1,286	1,273	1,364	1,299
Methylene blue reduction time, minutes	5.1	4.8	4.6	4.7

Each diluted semen sample was shipped to 61 different inseminators. These men used 1.0 ml. of the diluted semen for each insemination and each morning examined the semen for motility before using it for insemination that day. Few inseminations were made with fresh semen. The bulk of the inseminations were made on the second, third, and fourth days following collection.

RESULTS

The methods used for determining the results of insemination were discussed earlier (1, 2). The 5-month non-returns to service were considered as conceptions. The complete results are given in table 2. The highest average efficiency of conception was obtained with the highest dilution rate, 1:100. However, the small differences shown between dilution rates were not significant either when the percentage of conceptions for each ejaculate was used for the analysis of variance, or when the analysis of covariance using services as the independent variable and conceptions as the dependent variable was made. The average number of spermatozoa used for insemination at each of the dilution rates was as follows: 1:40, 31,361,000 spermatozoa; 1:60, 20,865,000; 1:80, 16,836,000; and 1:100, 12,861,000 spermatozoa per 1.0 ml. diluted semen.

The statistical analysis indicated highly significant differences between

TABLE 2

Fertility of the diluted semen by dilution rates

	Ratio of semen to diluter				Total	Non-returns %
	1:40	1:60	1:80	1:100		
Total services	1331	1386	1501	1547	5765	
Non-returns after breeding						
Avg. 45 days	914	938	1038	1086	3976	69.0
Avg. 75 days	807	827	923	966	3523	61.1
Avg. 105 days	760	772	868	914	3314	57.5
Avg. 135 days	740	738	836	885	3199	55.5
Avg. 165 days	722	725	820	870	3137	54.4
5-month non-returns, %	54.2	52.3	54.6	56.2		

the fertility levels of the bulls. This result was to be expected as bulls vary in their inherent level of fertility. Also, there was a highly significant interaction between bulls and dilution rates. This interaction showed no trend, but was a result of the variations in semen quality among the 4 samples from each bull. A careful study of the results from individual bulls shows no tendency for any one of them to decrease in fertility consistently with each dilution rate.

The results of this experiment show that bull semen of satisfactory quality may be diluted as high as 1:100, and that less than 0.01 ml. of semen in the 1.0 ml. of diluted material used for insemination, containing an average of about 13 millions of spermatozoa, will give results as satisfactory as lower rates of dilution. The conditions to which the diluted semen were subjected were as rigorous as are generally found in artificial breeding units. These conditions involve shipping of semen to all inseminators and competition for use by the inseminator between the semen in this experiment with other less dilute semen from other bulls shipped on the same days.

The data in table 2 is arranged to show the numbers of cows that are apparently "settled" an average of 45, 75, 105, 135, and 165 days after insemination. In the right hand column of the table the percentage of apparent conceptions after each interval of time is shown. These data show an important fact observed in all records studied in this work. Apparently many artificially inseminated cows show delayed return to heat. The results reported here are typical of many studied by the author. Whether or not the cows showing delayed estrus after insemination actually conceived, or abortion resulted is not known. Nor is it clear whether or not these results are actually different than those found in natural service. Detailed evidence on this question would be desirable.

SUMMARY

In an investigation involving 5,765 inseminations no difference in fertility was found between dilution rates of 1:40, 1:60, 1:80, or 1 part of bull semen to 100 parts of the egg yolk-citrate diluent. At the highest rate of dilution on the average 12,836,000 spermatozoa were introduced in each 1.0 ml. of diluted semen used for insemination.

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FROZEN HOMOGENIZED MILK. 1. EFFECT OF FREEZING AND STORAGE TEMPERATURE ON THE PHYSICAL CHARACTERISTICS OF HOMOGENIZED MILK

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During World War II frozen homogenized milk was used to supply fresh milk to patients on hospital ships. Usually this milk was very acceptable when thawed after having been stored in a frozen condition for 3 months and frequent reports indicated that its acceptability was good after 6 to 9 months of storage. Instances occurred, however, where the milk was unacceptable when thawed after storage for less than 3 months. In these instances either a separation of the caseinate system had occurred or the milk had developed an oxidized flavor. No adequate explanation was available for these conditions, since the milk apparently was processed, frozen, and stored in the same manner as the milk that was acceptable.

To determine why some milk was acceptable after prolonged storage in a frozen state, while similar milk apparently treated in the same manner was unacceptable after a short storage period in a frozen state, experiments were conducted at the Veterinary Laboratory, Medical Department Professional Service Schools, Army Medical Center, Washington, D. C., to study the effect of freezing and storage temperatures on the physical, chemical, and bacteriological characteristics of homogenized milk.

A large proportion of the information reported in the literature on the effect of freezing on milk is based on observations with unhomogenized milk. However, Cvitl (1) reported that the tendency to separate, of almost all the milk constituents under the same experimental conditions, is clearly less and the fluctuations from the average values smaller, in homogenized milk than in normal milk. Grayson (2) reported that homogenized milk packaged and frozen under vacuum remained of excellent quality, and that comparison with fresh milk indicated there was very little, if any, change in the frozen product. Webb and Hall (3) state that slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion. Thurston (4) states that homogenization, prolonged agitation at low temperature, and freezing and thawing of milk reduce or eliminate its susceptibility to oxidized flavor development. Bell (5) reported that homogenization prevents the formation of an undesirable layer of fat on the surface of condensed milk that

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has been frozen and aids in retarding changes which are indicated by an oxidized flavor. Roadhouse and Henderson (6) reported that pasteurized homogenized milk frozen in cans vacuum sealed under 23 inches of vacuum at 25° F. with agitation and stored at - 5° F. for 6 weeks had a good flavor, but not quite equal to the product when fresh; and Trout (7) states that a slightly greater percentage of homogenized than unhomogenized milk froze in a given period at 0° F.

PROCEDURE

The milk used in this work was obtained from a commercial dairy in the Washington, D. C., area where it was packaged in quart containers. An effort was made to obtain milk of uniform quality by selecting cases of milk as they came from the filler. The milk had a butterfat content slightly under 4 per cent. It was properly homogenized, as shown by the test for homogenized milk recommended in the United States Public Health Service Milk Ordinance and Code (1939).

The milk was frozen in the original containers and stored in constant-temperature freezers. The frozen milk was thawed at room temperature. The visual appearance of the milk when thawed was noted and the degree of separation was determined by pouring 50-cc. portions from the quart samples, after thoroughly mixing the sample, into conical tubes and centrifuging. This procedure was the same as that used for determining the solubility index of dried milk and is referred to as sediment in this paper. Flavor determinations were made, after the milk was completely thawed out, by a panel of three men all of whom had had previous experience in milk judging.

EXPERIMENTAL RESULTS

Freezing and storing at constant temperature. To determine the effect of freezing and storing milk at a constant temperature, milk was frozen and stored at - 10° C. (14° F.), - 32.8° C. (- 27° F.), and - 40° C. (- 40° F.). Table 1 shows the results obtained.

Table 1 indicates that freezing and storage at constant temperature can be applied to homogenized milk satisfactorily. For long storage periods, however, very low temperatures should be used. Samples held at - 10° C. showed a slightly flat flavor at the end of 21 days, whereas, samples held at - 32.8° C. and - 40° C. were normal for at least 115 days. Of the 14 samples held at these two lower temperatures one had a slightly flat flavor and one had a slightly oxidized flavor. No reason was found for these changes.

Freezing homogenized milk and storing at lower temperatures. To determine whether milk could be frozen at one temperature and stored at a lower one, four samples of homogenized milk were frozen and stored for 9 days at - 10° C. (14° F.). Then two of the samples were moved to storage at – 32.8° C. (– 27° F.) and two to storage at – 40° C. (– 40° F.). Two other samples were frozen and stored for 4 days at – 32.8° C. (– 27° F.) and then moved to storage at – 40° C. (– 40° F.). Results are shown in table 2.

Table 2 shows that in all instances the appearance remained normal. This was confirmed by low sediment readings. No abnormal flavors other than garlic were noted. Section B of table 2 shows that these samples also had a normal appearance and flavor when thawed at the end of 58 and 89 days respectively. Table 2, therefore, indicates there are no physical effects on homogenized milk frozen at one temperature and moved to a lower temperature.

TABLE 1

Effect of storing frozen homogenized milk at constant temperature

No. of samples	Total storage time (days)	Appearance when thawed	Flavor	Sediment (cc.)
(A) Frozen and held at – 10° C.				
2	21	Normal	Slightly flat	
1	58	Normal	Flat	0.45
2	89	Slight separation	Flat	1.50
3	100	Separated		6.00
(B) Frozen and held at – 32.8° C.				
1	30	Normal	Normal	
1	40	Normal	Normal	
1	58	Normal	Normal	0.08
1	89	Normal	Slightly flat	0.03
2	100	Normal		0.08
6	115	Normal	Normal	0.10
(C) Frozen and held at – 40° C.				
1	51	Normal	Normal	0.10
1	82	Normal	Slightly oxidized	0.03

Freezing homogenized milk and storing at higher temperatures. To further determine the effect of temperature changes on the physical characteristics of frozen homogenized milk, samples were frozen and held at one temperature and then stored at a higher temperature. Results are shown in table 3.

Table 3 indicates that homogenized milk should not be frozen and held at one temperature and then stored at a higher temperature. The only exception in table 3 was the milk frozen and stored at – 40° C. for 1 day and then moved to – 32.8° C., which was still normal at the end of 82 days. This may be attributed to the small difference between the initial

TABLE 2

Effect of storing frozen homogenized milk at a temperature lower than the initial freezing and holding temperature

No. of samples	Subsequent temperature	Subsequent storage time (days)	Appearance when thawed	Flavor	Sediment (cc.)
(A) Frozen and held for 9 days at −10° C.					
1	−40° C.	49	Normal	Very slight garlic*	0.15
1	−40° C.	80	Normal	Very slight garlic*	0.08
1	−32.8° C.	49	Normal	Trace garlic*	0.10
1	−32.8° C.	80	Normal	Very slight garlic*	0.03
(B) Frozen and held for 4 days at −32.8° C.					
1	−40° C.	54	Normal	Normal	0.08
1	−40° C.	85	Normal	Normal	0.08

* These samples were taken at a time when garlic was prevalent in the area and in view of the other samples it seems apparent that the garlic flavor existed in the milk prior to freezing.

TABLE 3

Effect of storing frozen homogenized milk at a temperature higher than the initial freezing and holding temperature

No. of samples	Subsequent temperature	Subsequent storage time (days)	Appearance when thawed	Flavor	Sediment (cc.)
(A) Frozen and held for 1 day at −40° C.					
1	−32.8° C.	50	Normal	Normal	0.08
1	−32.8° C.	81	Normal	Normal	0.03
1	−10° C.	50	Normal	Oxidized	0.50
1	−10° C.	81	Separated	Oxidized	4.00
(B) Frozen and held for 5 days at −32.8° C.					
1	−10° C.	15	Normal		
1	−10° C.	46	Normal	Flat	0.45
2	−10° C.	77	Separated	Oxidized	3.25
1	−3° C.	15	Slightly separated		
1	−3° C.	100	Separated	Flat	1.6
(C) Frozen and held for 13 days at −32.8° C.					
1	−3° C.	100	Separated	Flat	1.5

freezing and storage temperature and the subsequent storage temperature. In those samples where the temperature difference was great, there were flavor changes. The flavor became either flat or oxidized. For the two samples frozen and stored at – 40° C. for 1 day and moved to – 10° C. the appearance was normal for at least 51 days, but had separated to the extent of 4.00 cc. of sediment in 82 days. Similar results were obtained when samples were frozen at – 32.8° C. for 5 days and moved to storage at - 10° C. However, samples stored at – 3° C. (26.6° F.) following freezing and storage at – 32.8° C. for 5 days showed a slight separation in 20 days. The sediment readings on the two samples held for 100 days at this temperature do not fully indicate the separation that took place in this milk. No explanation can be given for this but the sediment in these samples was in the form of very fine particles instead of being flaky as it was in the other samples.

Effect of exposing frozen homogenized milk to room temperature. To simulate conditions of transferring frozen milk from land storage to ship storage, samples were exposed for various lengths of time at room temperature, *i.e.*, 23° C. (73.8° F.). Table 4 shows the effect of freezing and holding homogenized milk at different temperatures for different lengths of time, exposing it to room temperature for 30 minutes to 4 hours, then storing it at – 10° C.

Table 4, A, B, C, indicates as does table 1 that – 10° C. is too high a temperature for freezing and storing homogenized milk over long periods of time. Homogenized milk frozen and stored at this temperature and exposed to room temperature during its storage period was separated when thawed. The amount of separation, as confirmed by sediment readings, increased with the increase in exposure time and with the increase in the storage period. A comparison of the sediment readings obtained from frozen homogenized milk held at a constant temperature of – 10° C. (table 1) with those obtained where the milk was held at this temperature but exposed to room temperature (table 4, A, B, C,) shows that exposure to room temperature increased the degree of separation. Where the samples were stored for 58 days at a constant temperature (table 1) there was a sediment reading of 0.45 cc. as compared to 0.70, 2.30, 0.75 and 3.60 cc. where the milk was exposed to room temperature for ½ hour, 1 hour, 2 hours, and 4 hours, respectively (table 4). After 89 days storage the sediment reading increased from 1.50 cc. (table 1) to 2.75, 3.00, 3.50 and 9.50 cc. when exposed for ½ hour, 1 hour, 2 hours, and 4 hours, respectively (table 4). Likewise after 100 days storage the sediment readings increased from 6.00 cc. (table 1) to 9.00, 11.00, 13.00, and 12.00 cc. when exposed for ½ hour, 1 hour, 2 hours, and 4 hours, respectively (table 4).

Table 4, D, shows that homogenized milk frozen and stored at – 32.8° C. for 5 days before exposure to room temperature, with subsequent storage at

TABLE 4

Effect of exposing frozen homogenized milk to room temperature and returning to storage

No. of samples	Length of exposure (hours)	Total storage time (days)	Appearance when thawed	Flavor	Sediment (cc.)
(A) Frozen and held at − 10° C. for 9 days, exposed to room temperature and then stored at − 10° C.					
1	½	58	Slight separation	Flat	0.70
2	½	89	Separated	Flat	2.75
1	1	58	Separated	Flat	2.30
1	1	89	Separated	Flat	3.00
1	2	58	Slight separation	Flat	0.75
1	2	89	Separated	Flat	3.50
1	4	58	Separated	Flat	3.60
2	4	89	Separated	Flat	9.50
(B) Frozen and held at − 10° C. for 21 days, exposed to room temperature, and then stored at − 10° C.					
1	½	100	Separated		9.00
1	1	100	Separated		11.00
1	2	100	Separated		13.00
(C) Frozen and held at − 10° C. for 30 days, exposed to room temperature, and then stored at − 10° C.					
1	4	100	Separated		12.00
(D) Frozen and stored at − 32.8° C. for 5 days, exposed to room temperature, and then stored at − 10° C.					
1	½	51	Normal	Slightly oxidized	0.15
2	½	82	Separated	Slightly oxidized	2.00
1	1	51	Normal	Slightly oxidized	0.10
1	1	82	Slight separation	Slightly oxidized	1.30
1	2	51	Normal	Slightly oxidized	0.50
1	2	82	Separated	Slightly oxidized	1.80
1	4	51	Separated	Slightly oxidized	4.20
2	4	82	Separated	Slightly oxidized	2.50
(E) Frozen and stored at − 32.8° C. for 5 days, then stored at − 10° C. for 20 days, exposed to room temperature, and then stored at − 10° C.					
1	½	104	Separated		12.00
1	1	104	Separated		13.00
1	2	104	Separated		13.00

– 10° C., when thawed was normal in appearance for exposure up to 2 hours and storage time up to 51 days. The milk exposed to room temperature for 4 hours, however, was separated. The flavor of the samples including those that were normal in appearance was slightly oxidized. A comparison of table 4, D, with table 3, B, indicates that where homogenized milk is frozen and held at one temperature and then stored at a higher temperature, exposing it to room temperature does not greatly increase the degree of separation over that caused by a change in storage temperature but does affect the flavor of the milk.

Table 4, E, shows that samples which were frozen and stored at – 32.8° C. for 5 days, then stored at – 10° C. for 20 days before exposure to room temperature, and then stored at – 10° C. for an additional 79 days, were badly separated after thawing and all sediment readings were very high.

SUMMARY

Homogenized milk remained normal when frozen and stored at a constant temperature. The length of time it remained normal in the frozen state depended on the freezing and storage temperature.

Homogenized milk frozen and stored at – 10° C. (14° F.) had a slightly flat flavor when it was thawed at the end of 21 days. At the end of 89 days the flavor was definitely flat and slight separation had occurred. Homogenized milk frozen and stored at – 32.8° C. (– 27° F.) remained normal for 115 days and there were indications that it would remain normal for a longer period. There were also indications that homogenized milk frozen and stored at a still lower temperature, *i.e.*, – 40° C. (– 40° F.) would remain normal longer than milk frozen and stored at – 32.8° C. (– 27° F.).

Homogenized milk frozen and held at one temperature was moved to a lower temperature without causing any abnormalities to develop, but when it was moved to a higher temperature it became abnormal in appearance and flavor. Homogenized milk frozen at – 32.8° C. (– 27° F.) and subsequently stored at a lower temperature, *i.e.*, – 40° C. (– 40° F.), was still normal when thawed at the end of 89 days of total storage time. However, milk frozen at – 40° C. (– 40° F.) and then stored at a higher temperature, *i.e.*, – 10° C. (14° F.), was abnormal both in appearance and flavor when thawed if the total storage time had exceeded 50 days.

When frozen homogenized milk was exposed to room temperature for ½ hour, or more, at any time during its storage period with subsequent storage at a temperature higher than the initial storage temperature, its flavor when thawed was either flat or oxidized even though the milk was normal in appearance.

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THE PLACENTAL TRANSMISSION AND FETAL STORAGE OF VITAMIN A AND CAROTENE IN THE BOVINE

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The literature records several references (1, 3, 5, 7) that state there is little or no reserve of vitamin A in the newborn calf. However, it is known that a lack of vitamin A or its precursor, carotene, in the pregnant cow's diet may result in a stillborn, blind, or weak calf. Obviously, a sufficiency of this vitamin must traverse the placental barrier to provide for the normal fetal development. However, the factors involved in the placental transmission and fetal storage of vitamin A have not been explored. The results of Braun and Carle (2), although complicated by pathological conditions, suggest that the vitamin A content of the bovine fetal liver may be influenced by the maternal diet.

The relationship of the quantitative and qualitative factors of the maternal diet to the postnatal health of the newborn calf has received only limited attention. As a part of a broad project to investigate this relationship, data have been obtained regarding the effect of the prepartum diet on carotene and vitamin A storage in the newborn calf.

EXPERIMENTAL

Twenty-nine pregnant Holstein and four pregnant Guernsey heifers with similar management history were divided into four dietary groups approximately 60 days prior to calving. Each animal received a 12 per cent protein fitting ration composed mainly of cereal grains. The diets of the four groups in addition to the fitting ration were made up as follows: low carotene diet (S) of wheat straw, normal dry cow diet (N) of U. S. No. 1 timothy-clover mixed hay and corn silage, high carotene diet (C) of the same roughages as the N diet supplemented with one million I.U. daily of carotene, and a high vitamin A diet (A) of the same roughages as the N diet supplemented with one million I.U. daily of vitamin A in shark-liver oil. The carotene supplement was a commercial concentrate[1] containing 50,000 U.S.P. units per gram derived from vegetable sources. The vitamin A supplement was a high potency shark-liver oil[2] containing 41,000 I.U. per gram. The carotene content of the mixed hay and the corn silage as fed was 11.6 and 5.0 micrograms per gram, respectively. Unfortunately a shortage of labor made it impractical to record daily feed intakes.

Plasma carotene and vitamin A analyses were made on three successive days at the beginning of the experimental period (60 days before calving)

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[1] Purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

[2] Supplied by National Oil Products Co., Patterson, N. J.

and again about 18 days prior to the calving date. Because of the large daily variation it was believed that the average of three consecutive days would be more representative of the plasma levels. The blood plasma of the newborn calves was analyzed for vitamin A and carotene prior to colostrum ingestion. The method of Kimble (6) was used to determine plasma vitamin A and carotene. Several calves from the cows in each dietary group were killed at birth and liver storage of vitamin A and carotene determined according to the extraction procedure of Davies (4).

RESULTS

Plasma carotene and vitamin A of the pregnant cows. Table 1 shows the plasma carotene and vitamin A of the pregnant cows at approximately 60 days and 18 days before calving. The mean values accompanied by their standard errors are presented for each dietary group. Although there was considerable individual variation, the means for carotene and vitamin A were quite high at the beginning of the experimental feeding period.

The mean differences between the 60-day and 18-day values are a measure of the effects of the different dietary regimens on the plasma carotene and vitamin A. It does not seem likely that the usual gestational decrease in plasma carotene and vitamin A was a factor since this effect has been reported (8, 12, 13) to occur mostly during the two weeks prior to parturition. It is not known whether large daily intakes of vitamin A will prevent this decline. However, Lund (10) reported that the daily administration of 25,000 I.U. of vitamin A to pregnant women was ineffective.

The mean plasma carotene and vitamin A of group S was significantly lower than the means of the other groups 18 days before calving. The decline in plasma carotene and vitamin A of the N group is typical of the usual trend when cows are placed on barn feeding following a period on pasture. It should be pointed out that all of the experimental animals were on pasture previous to the start of the experiment. Oral administration of one million I.U. of carotene daily to the C group maintained the plasma carotene level, but did not prevent a slight decline in vitamin A over the 42-day period. It is of interest that the mean plasma vitamin A at 18 days before calving was highest for group A and lowest for group S. The difference between these means was highly significant. The difference between the other means was not significant statistically. It seems clear from these results that in maintaining the plasma levels of carotene and vitamin A of the dry cow, there is no advantage in supplementing a good practical ration with additional carotene or vitamin A. However, these results show the value of feeding the dry cow a ration of natural feedstuffs high in carotene.

Plasma carotene and vitamin A of the newborn calf. The data in table 2 show the relationship of the prepartum diet of the cow to the plasma carotene and vitamin A of the newborn calf. No significant differences were observed

TABLE 1

The maternal plasma carotene and vitamin A 60 days and 18 days before calving

Animal number	Plasma carotene			Plasma vitamin A		
	60 days	18 days	Change	60 days	18 days	Change
			Low carotene diet (S)			
	γ/100 ml.	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*
R-26	851	138	-713	36	17	-19
3	484	101	-383	23	19	- 4
R-86	879	91	-788	37	21	-16
8	647	101	-546	32	17	-15
12	385	65	-320	42	15	-27
13*	1406*	569*	-837*	27*	16*	-11*
17	429	112	-317	29	17	-12
R-95	473	82	-391	34	19	-15
24	304	45	-259	21	17	- 4
Mean	557 ± 66	92 ± 9	-465 ± 69	32 ± 0.8	17 ± 0.6	-14 ± 0.3
			Normal diet (N)			
	γ/100 ml.	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*
1	537	297	-240	40	18	-22
7	669	336	-333	35	45	+10
14	235	195	- 40	52	30	-22
R-74	846	624	-222	41	45	+ 4
23	406	239	-167	33	25	- 8
34						
Mean	539 ± 105	338 ± 76	-200 ± 48	40 ± 3.9	32 ± 5	-8 ± 7
			Normal diet plus carotene (C)			
	γ/100 ml.	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*
4	520	511	- 9	37	25	-12
5	409	408	- 1	36	33	- 3
9	596	488	-108	29	23	- 6
10	764	276	-488	27	31	+ 4
18	301	352	+ 51	28	22	- 6
29*	618*	474*	-144*	42*	52*	+10*
30*	364*	1313*	+949*	24*	18*	- 6*
31	173	284	+111	43	33	-10
32	254	276	+ 22	36	32	- 4
33	217	243	+ 26	38	34	- 4
Mean	404 ± 65	355 ± 36	-50 ± 66	34 ± 2	29 ± 2	-5 ± 2
			Normal diet plus vitamin A (A)			
	γ/100 ml.	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*	*γ/100 ml.*
2	708	217	-491	30	25	- 5
R-64	1067	284	-783	44	27	-17
6	356	282	- 74	29	39	+10
16	204	291	+ 87	28	32	+ 4
20*	962*	544*	-418*	39*	45*	+ 6*
22	142	142	0	39	43	+ 4
26	303	155	-148	37	50	+13
27	373	106	-267	28	40	+12
Mean	450 ± 124	211 ± 29	-239 ± 116	33 ± 2	36 ± 10	+3 ± 4

* Denotes Guernsey cows not considered in average.

in the mean plasma carotene of newborn calves from the four dietary groups. The mean of group A was the highest while the mean of group C was the lowest. There was no correlation between the levels of maternal and new-

TABLE 2

The effect of maternal diet upon the plasma carotene and vitamin A of the newborn calf

Dam's number	Plasma of newborn calf	
	Carotene	Vitamin A
	Low carotene diet (S)	
	γ/100 ml.	*γ/100 ml.*
R-26	4.5	5.1
3	0.8	1.0
8	1.5	1.4
12	1.5	2.6
13	6.0*	2.9*
17	1.9	0.6
R-95	1.7	3.1
24	0.8	1.5
Mean	1.8 ± 0.5	2.2 ± 0.6
	Normal diet (N)	
	γ/100 ml.	*γ/100 ml.*
1	1.6	1.5
7	1.5	1.5
14	1.7	1.6
R-74	0.8	3.3
34	1.0	2.5
Mean	1.3 ± 0.2	2.1 ± 0.4
	Normal diet plus carotene (C)	
	γ/100 ml.	*γ/100 ml.*
4	1.5	3.9
5	0.8	5.2
9	1.0	8.1
10	1.5	5.2
18	1.6	4.1
29	0.8*	0.8*
31	1.0	3.0
32	1.5	0.8
33	0.0	2.8
Mean	1.1 ± 0.4	4.6 ± 0.8
	Normal diet plus vitamin A (A)	
	γ/100 ml.	*γ/100 ml.*
2	6.7	9.3
6	1.5	20.0
16	2.7	7.3
20	2.2*	6.6*
22	1.2	8.3
26	0.0	6.7
27	1.0	4.2
Mean	2.2 ± 1.0	9.3 ± 2.2

[*] Guernsey omitted from average.

The effect of maternal diet upon the fetal storage of carotene and vitamin A in the liver of the newborn calf

Dam's number	Birth weight of calf	Weight of calf's liver	Liver storage				Total I.U. in liver‡
			Carotene	Vitamin A	Total carotene	Total vitamin A	
			Low carotene diet (S)				
	lbs.	*gms.*†	γ/*gm.*†	γ/*gm.*†	γ	γ	
3	100	666	0.014	0.18	9.32	119.88	492
13	60*	575*	0.32*	0.085*	184.00*	48.88*	502*
17	78	720	0.10	0.08	72.00	57.60	432
R–95	105	856	0.13	0.019	111.28	16.26	256
Mean	94	750 ± 61	0.081 ± .03	0.093 ± .047	64.2 ± 29.7	64.5 ± 49.2	393 ± 71.9
			Normal diet (N)				
	lbs.	*gms.*†	γ/*gm.*†	γ/*gm.*†	γ	γ	
7	87	675	0.136	0.33	91.80	222.75	1,044
R–74	122	940	0.140	1.74	131.60	1,635.60	6,760
23	88	733	0.090	0.096	65.97	70.37	390
34	78	707	0.107	0.39	75.65	275.73	1,226
Mean	94	764 ± 59	0.118 ± .013	0.64 ± .37	91.2 ± 14.4	551.1 ± 364.5	2355 ± 1481
			Normal diet plus carotene (C)				
	lbs.	*gms.*†	γ/*gm.*†	γ/*gm.*†	γ	γ	
4	93	653	0.085	0.22	55.51	143.66	663
29	67*	490*	0.21*	0.22*	102.90*	107.80*	602*
30	76*	430*	0.43*	0.14*	184.90*	60.20*	548*
31	97	850	0.24	0.49	204.00	416.50	2,005
32	94	846	0.52	0.36	439.92	304.56	1,948
33	87	655	0.19	0.18	124.45	117.90	655
Mean	93	687 ± 77	0.26 ± .04	0.31 ± .08	205.9 ± 83.7	245.6 ± 70.4	1.319 ± 380
			Normal diet plus vitamin A (A)				
	lbs.	*gms.*†	γ/*gm.*†	γ/*gm.*†	γ	γ	
6	100	810	0.14	25.45	113.40	20,614.50	85,011
16	102	729	0.11	4.31	80.19	3.141.99	12,705
20	65*	419*	0.22*	25.42*	92.18*	10,650.98*	43,874*
22	110	766	0.11	43.50	84.26	33,321.00	133,374
26	105	988	0.57	44.70	563.16	44,163.60	177,735
27	77	640	0.14	30.10	89.60	19,264.00	77,060
Mean	99	787 ± 56	0.21 ± .09	29.61 ± 7.35	186.1 ± 94.4	24,101.1 ± 6936	97.177 ± 881

*** Guernsey omitted from average. † Wet basis. ‡ Assuming 0.6 micrograms carotene = one I.U. of vitamin A.**

born plasma carotene, nor was there any demonstrable relation of maternal diet to the plasma carotene of the newborn.

The mean plasma vitamin A values of groups S and N are comparable to the values for normal calves reported by Moore and Berry (11). However, the mean value for group C was twice as great as for groups S and N, and the mean for group A was fourfold greater than normal. Statistically significant differences were found between the mean of the C or A groups and the means of the other groups. There was no correlation between the plasma A and carotene of the newborn calf.

According to Lund (10), similar studies with humans gave somewhat different results. He reported that fetal plasma levels of vitamin A were completely independent of maternal levels and could not be elevated even when the mother was given enormous doses (330,000 I.U.) of vitamin A daily before delivery. However, he reported that fetal carotene values were directly related to those of the mother and to the prenatal diet. In contrast, the results reported herein indicate that only the vitamin A in the plasma of the newborn calf may be elevated by the prenatal diet of the cow.

Fetal liver storage of carotene and vitamin A. Table 3 shows the individual carotene and vitamin A content per gram of liver in the newborn calf for each of the four diets, the total micrograms of carotene and vitamin A, and the total vitamin A potency per liver.

The effects of maternal diet on fetal liver storage of carotene and vitamin A were quite evident. Carotenoids were present in significant amounts in the livers of all calves contrary to the reports of other investigations. While the amount per gram of liver tissue is a measure of concentration, it does not represent a true picture of the carotene reserves. It should be noted that the mean total liver storage of carotenoids in terms of micrograms was 64.2 for group S, 91.2 for group N, 205.9 for group C, and 186.1 for group A. These results show that carotene is not only stored in the bovine fetal liver, but that the amount stored is in direct relation to the carotene content of the maternal diet. The considerable variation observed among individual calves emphasizes the importance of making several observations.

The effects of the cow's prepartum diet on the storage of vitamin A in the liver of her newborn calf are shown in table 3. For the low carotene (S) group, the mean value was 0.093 micrograms per gram of fresh liver tissue while the mean for the normal diet (N) group was 0.64. The reason that the mean value of 0.31 for the carotene-supplemented (C) group was lower than the N group was due to the large values obtained for the calf from cow R–74. Placental transmission and fetal storage was tremendously greater in the calves of group A from the vitamin A supplemented dams. The mean for this group was 29.61 micrograms per gram. Total liver reserves of vitamin A, *per se,* were 64.5 for group S, 551.1 for group N, 245.6 for group C and 24,101.1 micrograms for group A.

In the final column of table 3 we have listed under the heading of total I.U.[3] the vitamin A reserves in the liver of the newborn calves. The mean values were 393 for group S, 2,355 for group N, 1,319 for group C, and 97,177 for group A. Mention should be made of the fact that the calf from cow R-95 was blind at birth. Since this calf had a total liver storage of only 256 I.U., the blindness may have been due to insufficient vitamin A. If we assume complete utilization and a daily requirement of 10,000 I.U. per 100 pounds of body weight as suggested by Lewis and Wilson (9), the calves from the vitamin A supplemented cows were born with a storage equal to ten days' requirement of vitamin A.

No differences were observed in the mean birth weights of the calves, nor was there any relation between body weight and weight of the fresh liver.

DISCUSSION

It is worthy of note that the daily addition of a million I.U. of vitamin A to the usual dry cow ration either depressed or did not prevent a marked decline in the plasma carotene of the cows in group A. Additional data are being obtained on this problem and on the effect of diet on the gestational decrease of maternal plasma vitamin A.

Apparently the plasma carotene of the newborn calf is independent of the maternal plasma level and diet. This inference does not preclude placental transfer of carotene as indicated by the increased liver storage of carotene in the newborn calves of group C. These calves had slightly less vitamin A storage in the liver, yet their plasma vitamin A was higher than normal newborn calves. These facts together with the increased maternal values for plasma carotene and no apparent increase in plasma vitamin A are indicative of some fetal synthesis of vitamin A.

The variation of the results among the individual cases subjected to fairly uniform treatment suggests that factors in addition to diet are involved in the placental transmission and fetal storage of vitamin A and carotene. Although this variation tends to nullify the statistical differences among the means, the importance of the results is not diminished.

To our knowledge the data here presented represent the first clear demonstration that, in the normal bovine, the prepartum diet may influence markedly the level of both vitamin A and carotene in the plasma and liver of the new-born calf. In contrast to the present commonly accepted viewpoint, it has been shown that calves need not be deficient in vitamin A at birth. The question warrants further study as to whether or not feeding extra vitamin A to the pregnant cow will benefit the newborn calf. Clearly it would seem more economical to feed vitamin A directly to the newborn calf unless increasing the fetal reserve can be shown to give superior performance.

[3] Assuming 0.6 micrograms carotene = one I.U. of vitamin A.

SUMMARY

Four different rations were fed to 29 Holstein and 4 Guernsey heifers during the last 60 days of their gestation periods. The rations were: wheat straw plus a concentrate mixture, a normal fitting ration of concentrates, hay and corn silage, a normal ration plus one million I.U. of carotene daily, and the normal ration plus one million I.U. of vitamin A daily.

No significant differences were observed in the mean plasma carotene of the newborn calves from the four dietary groups. However, the plasma vitamin A of the newborn calves from the carotene supplemented cows was twice that of the normal group, while the vitamin A supplemented group showed a fourfold increase.

Carotenoids were present in significant amounts in the livers of all newborn calves and varied directly with the carotene content of the maternal prepartum diet.

The addition of one million I.U. of vitamin A daily to the normal ration of pregnant cows resulted in an average total fetal liver storage of 97,177 I.U. of vitamin A.

Evidence has been presented that the prepartum diet of the normal bovine may influence markedly the vitamin A and carotene reserves of the newborn calf.

Addendum—

Since the completion of the study presented herein, Wise and associates (14) have reported limited data indicating that the addition of vitamin A to the late gestation diet of the cow augmented significantly the vitamin A concenration in the blood and livers of the newborn calf.

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DIFFERENTIAL NITROGEN RETENTION FROM CASEIN, LACTALBUMIN, AND SOY PROTEIN, AND HYDROLYSATES THEREFROM[1]

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Protein utilization by the body involves three important features: the inherent amino acid content of the protein, the degree to which its components may be absorbed and the retention and utilization after absorption. Methods for the integration and evaluation of these features include determination of the amino acids by analytical methods, determination of digestibility, and nitrogen retention with experimental animals.

From a theoretical point of view a protein hydrolysate should limit variations in digestibility. Assuming that the method of preparation has not destroyed essential amino acids the first phase of digestion may be considered to have been accomplished before ingestion. Conservation in the efficiency of protein metabolism through the expedient of predigestion does not assume, however, superiority of retention and utilization because of increased absorption.

The hydrolytic process is essentially one of degradation, therefore, a protein hydrolysate may not necessarily be the full equivalent of the starting material. Destruction of tryptophane by acid hydrolysis and the destruction of cystine and methionine by alkali are well known. Our immediate interest, however, concerns enzymatic hydrolysis simulating in principle, protein cleavage within the body. The literature has not revealed extensive studies designed to show the relative biological efficiency of natural proteins and their hydrolysates. Aside from a more rapid and possibly greater degree of absorption, there is no reason to presume that a hydrolysate has a greater biological value than the protein from which it was derived. Inasmuch as our unpublished data have shown that the amino acids resulting from the primary enzymatic cleavage of protein may be degradated to their fatty acid components by further enzymatic action, we have undertaken the present comparative study using three common proteins and their primary hydrolysates in recognition of the possibility of a destructive action depending upon the method of preparation. The proteins used for this study were casein, lactalbumin and a protein derived from soy bean. Each of these products could be readily prepared in purified form suitable for the biological studies and efficient preparation of the hydrolysate.

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EXPERIMENTAL

The protein in each instance was prepared in a manner designed to yield a product dispersible in water at a pH below neutrality. The casein as used in the experimental diets as well as that used for the enzymatic treatment was a purified di-calcium caseinate containing 1.72 per cent of bound calcium. At no time was the protein subjected to an alkalinity greater than pH 7.05 or an acidity stronger than pH 4.6. For the preparation of the hydrolysate a 10 per cent concentration of the dispersed caseinate was subjected to the action of a proteolytic enzyme for about 3 hours. The amino nitrogen as measured by formol titration was 28.4 per cent.

For the lactalbumin studies two unhydrolyzed products were used in comparison with the hydrolysate. One was a thoroughly washed, insoluble, iso-electric lactalbumin and the other a dispersible sodium lactalbuminate. This was prepared from the iso-electric lactalbumin by dispersing the insoluble product in weak alkali under controlled time and temperature conditions designed to minimize the destructive effect of the alkali. Upon completion of the dispersion the alkalinity was reduced to pH 6.8–6.9. An 8 per cent concentration of this dispersion was subjected to the same proteolytic enzyme employed for the preparation of the casein hydrolysate for a period of 4 hours. The hydrolysate contained 31.8 per cent amino nitrogen.

The soy protein was derived from a high grade of defatted soy meal by initial precipitation of the protein at pH 5.3. This precipitate subsequently subjected to a plurality of washings was then dispersed in hydrochloric acid at pH about 1.9–2.0. The protein in colloidal solution was precipitated at pH 4.3 and subjected to a series of washings while progressively increasing the pH of the suspension to 5.1. An 8 per cent concentration of the suspended protein was partially dispersed at pH 5.8 and subjected to the action of the hydrolytic enzyme for a period of 6 hours. The hydrolysate contained 32.2 per cent amino nitrogen.

According to the manufacturer the proteins of the soy meal used for the isolation of the protein used in this study are slightly, if at all, denatured by heat; "the protein is as undenatured as the protein of raw soy beans with a water solubility of about 80 per cent." Soy protein similarly prepared from the same starting material has been shown to cause a 10-gram gain per week in white rats when comprising 16 per cent of the ration supplemented with choline (6).

The determination of the biological merits of the various proteins and hydrolysates involved the primary features of methods proposed by Mitchell (3), Mitchell and Carman (4), Cannon (2), and Allison and Anderson (1) with adaptations to meet the requirements of this particular study.

Mature white rats averaging in excess of 200 grams were furnished a depletion ration consisting of the following: Salt mixture, No. 40 (5), 4 parts; hydrogenated vegetable oil (Crisco), 3 parts; cod-liver oil, 2 parts;

Cellu flour, 4 parts; dextrin, 50 parts; lactalbumin, 1.5 parts; and sucrose, 35.5 parts. The following vitamin factors were provided as a 2.5 ml. supplement daily: Thiamin, 12.5 mcgs.; riboflavin, 10.0 mcgs.; pyridoxine, 10.0 mcgs.; calcium pantothenate, 50.0 mcgs.; and choline, 10.0 milligrams. The ration contained 0.193 per cent nitrogen. The animals were maintained on this diet for a period of 2½ to 3 months during which period loss of weight averaged approximately 25 per cent while maintaining a slightly positive daily nitrogen balance based upon urinary excretion only, averaging 2.3 milligrams. Following this extended depletion period the lactalbumin was eliminated from the ration and the animals continued on a nitrogen-free (0.068 per cent nitrogen) ration and the vitamin supplements for a period of 7 days. During this final depletion period the animals lost on an average of 8.8 per cent of their weight following the low nitrogen ration and an average loss of 32 per cent of their original weight. The slightly positive urinary nitrogen balance shifted to a negative balance averaging 21.7 milligrams per day.

At the end of the last depletion period the test proteins and hydrolysates were incorporated in the "nitrogen-free" ration at comparable nitrogen levels without regard to protein equivalency. A series of nitrogen levels varying from 0.193 to 2.173 per cent were provided during a continuous sequence of 4-day test periods at each level, the vitamin supplements remaining the same. The rations were fed *ad lib* in guarded containers preventing scattering and food consumption records were obtained daily. Four to six animals were used for each test material.

The animals were maintained in individual metal and screened bottom cages Urine and feces were collected daily on filter paper. The droppings were immediately dried to constant weight and composited for each rat for each four-day period. The urine absorbed on the filter paper and that which may have adhered to the removeable trays was recovered daily by three extractions with 2 per cent boiling sulphuric acid. In order to follow closely the effect of each test material, nitrogen determinations were made on the urine daily for each animal in most instances and for all animals through the transition period from a negative nitrogen balance to a well-established positive balance.

All nitrogen determinations were made by a semimicro method involving digestion in Folin tubes and subsequent distillation with circulating steam under slight pressure.

The plan of procedure permits the calculation of biological values of the test materials according to Mitchell (3) wherein endogenous fecal and urinary nitrogen are calculated from the values obtained during the per-test period on the nitrogen-free ration and again following the test period; such values for each product at each of the nitrogen levels are shown in table 1. It will be noted that in all instances the higher biological values are shown at the three lower nitrogen percentage levels decreasing progressively at the higher levels. This relationship indicates optimum efficiency of nitrogen utilization at the lower intake levels. The range of variations noted may be due, in part at least, to the calculated endogenous urinary nitrogen value. Our data indicate that the endogenous urinary nitrogen may vary depending upon the quality of the protein, especially under experimental conditions involving an extended depletion period. For example, striking evidence of variation in excretion of endogenous nitrogen was consistently evident in the case of the lactalbumin feedings. The endogenous urinary nitrogen as determined from the nitrogen-free ration was in all cases higher and in some instances twice as high as the total urinary nitrogen excreted when lactalbumin was included in the ration, especially at the lower or medium percentage levels. This relationship may arise from varying degrees of depletion of the dispensable protein reserve, labile protein reserve or possibly indispensable fixed body protein according to the concept of Whipple (7), since food intake during the standardization periods and the test periods at the lower nitrogen intake levels was substantially iso-caloric and adequate for basal metabolism requirements. For illustration, the calculated caloric requirement during the standardization periods averaged 25.66 calories per day per animal for the group receiving lactalbumin; the actual caloric intake was 33.08 calories per day. During the lactalbumin test periods at the lower nitrogen levels, the caloric requirement averaged 24.68 calories per day; the caloric intake was 32.66 calories per day.

TABLE 1

Biological values of lactalbumin, casein, and soy protein, and their hydrolysates at different nitrogen intake levels

% Nitrogen in ration	Biological value						
	Lactalb.	Dispersed lactalb.	Dispersed lactalb hydrolysate	Casein	Casein hydrolysate	Soy protein	Soy protein hydrolysate
0.193	83.4	100.0	96.7	92.4	92.7	78.8	69.0
0.386	100.0	100.0	96.6	91.2	99.2	71.4	74.6
0.773	100.0	99.9	98.9	90.1	89.9	68.7	68.4
1.165	100.0	87.5	92.5	80.6	85.6	54.0	62.0
1.584	89.8	82.5	79.2	76.6	72.3	51.6	55.8
2.162	76.6	47.4	70.1	60.0	64.6	43.6	47.8

In view of the above we have presented the data correlating nitrogen balance with absorbed nitrogen on a cumulative basis, no attempt being made to calculate the endogenous increment of the urinary nitrogen. These values are recorded in table 2 and figure 1. The nitrogen balance correlated with absorbed nitrogen assumes a striking linear relationship similar to that shown by Allison and Anderson (1). Also, the graph clearly shows the

TABLE 2

Establishment and degree of positive nitrogen balance caused by lactalbumin, casein and soy protein and their hydrolysates

(Grams/sq. m./day—cumulative basis)

Test material	% nitrogen in ration	% absorbed	Amount absorbed	Amount retained
			gm.	*gm.*
None	0.068		0.2300	0.6841 –
Lactalb.	0.193	64.75	0.7860	0.2557 –
"	0.386	79.41	1.2710	0.2537
"	0.777	94.14	3.1353	2.0187
"	1.166	96.91	6.4598	4.7028
"	1.586	96.89	10.6518	7.8965
"	2.151	95.06	15.2778	10.5190
None	0.068		0.1348	0.0927 –
Dispersed lactalb.	0.198	94.37	0.7491	0.0272 –
" "	0.389	95.17	1.3908	0.3099
" "	0.774	96.56	3.4054	1.8449
" "	1.168	95.67	5.6202	3.5627
" "	1.584	95.30	9.1868	5.9338
" "	2.163	95.99	12.7533	8.6694
None	0.068		0.1956	0.7826 –
Lactalb. hydrolysate	0.193	75.08	0.8059	0.2176 –
" "	0.386	90.25	1.2311	0.1224
" "	0.771	96.51	3.3498	1.5148
" "	1.160	96.36	6.4885	3.6597
" "	1.590	95.49	10.2677	6.5481
" "	2.164	95.61	14.3976	8.4251
None	0.068		0.2482	0.7909 –
Casein	0.193	88.61	0.7832	0.2773 –
"	0.386	88.48	1.4055	0.0510 –
"	0.774	96.01	3.3506	1.3120
"	1.154	97.66	6.7934	3.2764
"	1.593	98.23	12.5479	6.8392
"	2.171	95.69	17.9786	10.6189
None	0.068		0.2700	0.7315 –
Casein hydrolysate	0.193	80.81	0.9885	0.2795 –
" "	0.386	83.77	1.3667	0.1856 –
" "	0.773	90.12	2.9563	0.8107
" "	1.165	95.00	5.7399	2.5013
" "	1.584	95.39	8.9637	4.2433
" "	2.162	95.74	15.0303	7.6375
None	0.068		0.1429	0.9598 –
Soy protein	0.193	93.30	0.6988	0.1584 –
" "	0.386	96.45	1.4096	0.0558 –
" "	0.773	96.56	3.0823	0.6213
" "	1.163	96.31	5.5650	1.5636
" "	1.582	98.08	8.9643	3.0676
" "	2.164	96.99	12.9224	4.7472
None	0.068		0.3050	0.7602 –
Soy protein hydrolysate	0.193	72.46	0.9842	0.5589 –
" " "	0.386	84.91	1.4784	0.0499 –
" " "	0.770	93.59	2.9785	0.4747
" " "	1.153	95.86	5.3699	1.5954
" " "	1.576	96.44	8.7165	2.6308
" " "	2.173	95.31	13.6188	4.6058

comparative nitrogen retention from the various proteins, and from the individual proteins and their respective hydrolysates. This type of evidence correlated with the biological value as usually calculated seems to yield more comprehensive information regarding the test material than is possible from the determination of the biological value resulting from a single feeding level. These data show that the lactalbumin and its hydrolysate are measurably better than casein or its hydrolysate, both of these proteins being superior to the soy protein; also, the protein appears to be slightly better than its hydrolysate in each instance.

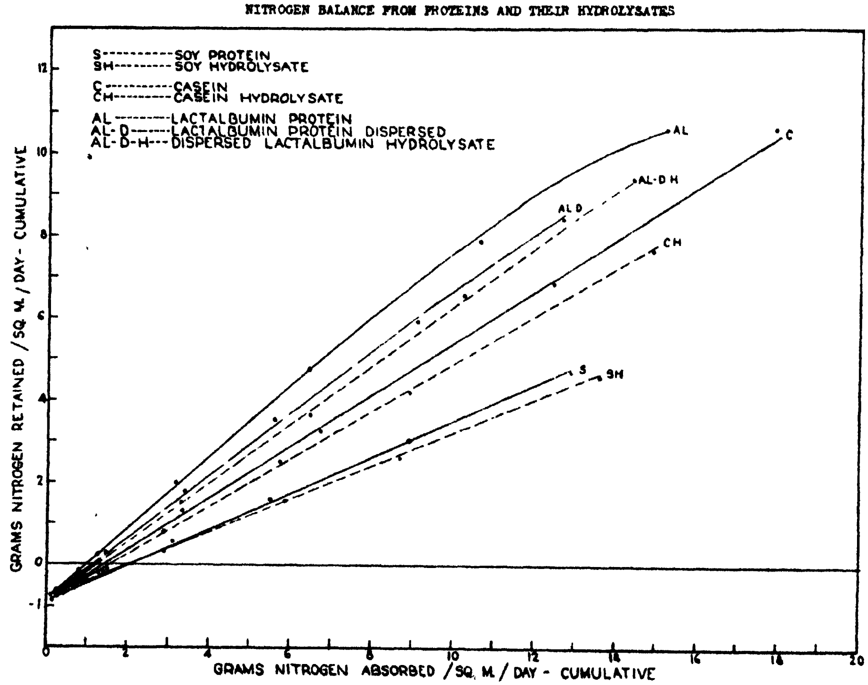

FIG. 1. Nitrogen balance from proteins and their hydrolysates.

Hemoglobin determinations and blood counts were made at the beginning and at the end of the 24-day test period. In all cases (table 3) there is an increase in both the hemoglobin and blood count. The data are not adequate for demonstrating a clear and conclusive differentiation between the different proteins and their respective hydrolysates.

Further evidence concerning the relative merits of the proteins and their hydrolysates is shown by the difference in nitrogen intake necessary to bring the animals to a state of equilibrium following the negative nitrogen balance existing at the end of the depletion period. Table 4 shows these values calculated as cumulative nitrogen intake per square meter of body area. The

TABLE 3

Hemoglobin and blood count regeneration as affected by the test proteins and hydrolysates during the 24-day test period

Material	Start of test period		End of test period	
	Hemoglobin	Blood count	Hemoglobin	Blood count
	gm./100 ml.	*cu. mm.*	*gm./100 ml.*	*cu. mm.*
Lactalb.	10.2	7,610,000	10.4	8,770,000
Dispersed lactalb.	9.9	7,550,000	10.9	8,130,000
Lactalb. hydrolysate	8.4	6,260,000	10.8	6,770,000
Casein	7.0	6,910,000	9.8	8,170,000
Casein hydrolysate	9.2	6,175,000	10.7	8,520,000
Soy protein	9.8	8,140,000	11.2	9,730,000
Soy protein hydrolysate	7.9	5,457,000	9.8	6,850,000

relationship between individual proteins and between the protein and its hydrolysate remains the same as shown by the curves in figure 1.

It is not presumed that analytical values are the only criteria for determining the relative merits of protein material. Weight gain is the most commonly used method for evaluating proteins, both qualitatively and quantitatively. Figure 2 correlates the loss or gain in weight with nitrogen retention for the entire 24-day test period involving the six different nitrogen intake levels. The graphs therefore, represent the overall results from the test products in overcoming the deficit in body nitrogen reserves and for reestablishment of weight gain. Lactalbumin and casein are shown to be superior to the soy protein. Gains of 10 to 12 or 15 grams per four-day period at the higher intake levels were not uncommon from the milk proteins after body reserves had been replenished at the lower intake levels.

Since high reserves of body protein are known to reduce nitrogen loss following hemorrhagic or traumatic shock, the influence of the three hydrolysates and a dispersible casein were used in a comparable manner designed to determine their effectiveness in reducing urinary nitrogen loss following severe shock. Four groups of mature rats were placed on the nitrogen-free ration previously described until a negative nitrogen balance

TABLE 4

Nitrogen intake required to establish equilibrium following the nitrogen depletion period

Material	Nitrogen balance at end of depletion	Nitrogen intake to establish equilibrium	
	gm./sq. m./day	*gm./sq. m.*	*gm./rat*
Lactalb.	0.6841 –	2.981	0.0954
Lactalb. hydrolysate	0.9826 –	3.203	0.1086
Casein	0.7909 –	5.400	0.1647
Casein hydrolysate	0.7315 –	6.456	0.1672
Soy protein	0.9598 –	3.780	0.1739
Soy protein hydrolysate	0.7602 –	8.994	0.2401

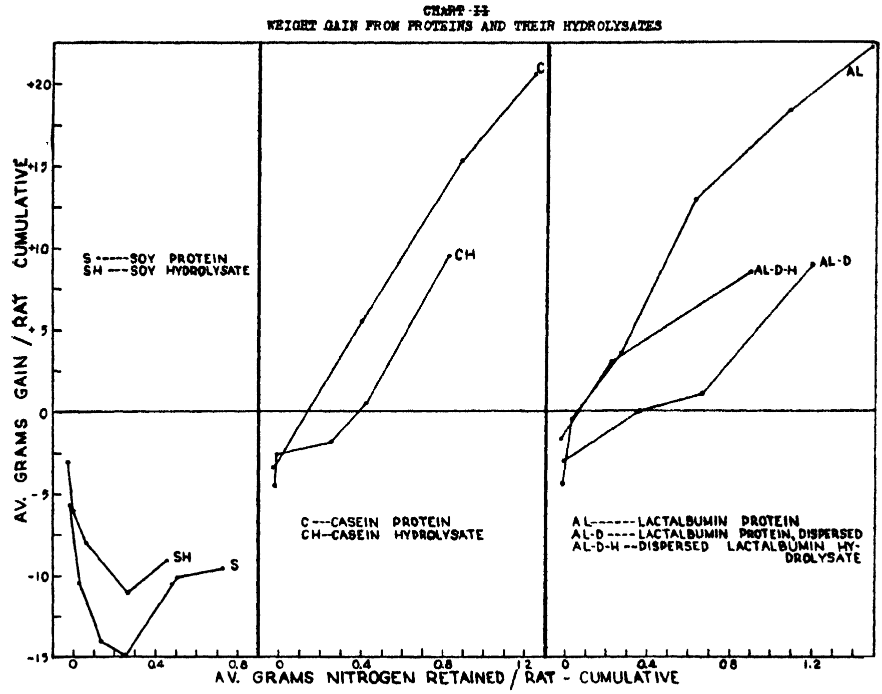

FIG. 2. Weight gain from proteins and their hydrolysates.

was well established. Two ml. of blood were withdrawn from the heart by hypodermic syringe and replaced with the test material dissolved in 2.0 ml. of physiological salt solution. The injection carried 15.9 mg. of nitrogen. The entire operation involved about five minutes. Since the object was to determine the comparative effectiveness of the test materials in reducing nitrogen loss, the urinary excretion was determined for the 48-hour period prior to injection and for a similar period following injection. As might be expected the highest percentage increase resulted following injection of the unhydrolyzed protein (table 5). The nitrogen excretion following injection

TABLE 5

Comparative loss of nitrogen before and after hemorrhagic shock and injection of protein hydrolysates

Material	Loss during 48 hr. before shock	Loss during 48 hr. after shock	% increase
	mg.	*mg.*	
Lactalb. hydrolysate	79.2	110.1	39.0
Casein hydrolysate	99.6	148.6	49.2
Dispersed casein	85.4	244.2	185.9
Soy protein hydrolysate	72.3	153.2	110.5

of the hydrolysates was substantially lower; the lactalbumin hydrolysate being most effective in reducing nitrogen loss, the soy hydrolysate being least effective, with the casein hydrolysate in an intermediate position approaching the effectiveness of the lactalbumin. Rate of nitrogen excretion for all groups returned to the pre-injection level after 5–7 days on the nitrogen-free ration.

SUMMARY

The various data illustrating different methods for the biological evaluation of the protein lead, generally speaking, to the same conclusions. The evidence clearly shows the superiority of lactalbumin over casein and the superiority of both casein and lactalbumin over soy protein. If a relative value of 100 was assigned to the lactalbumin, the value for the casein would be about 80 and that of the soy protein between 50 and 60. These general relationships have been indicated by published data from time to time, although the magnitude of the differences have been subject to variations depending upon the particular methods used.

Reference has been made to the linear relationship between nitrogen balance and absorbed nitrogen wherein variations in the slopes of the curves are of value as a means for determining the relative or absolute merits of the test materials. Such evidence shows that in each instance the original protein is slightly superior to its hydrolysate in respect to maintenance of a positive nitrogen balance. This relationship, however, is not consistently shown by the calculated biological values (table 1). The relatively greater difference between the iso-electric albumin and its hydrolysate may be accounted for by the intermediate step of peptization before hydrolysis. The difference between the peptized lactalbumin and the hydrolysate prepared direct therefrom is not of substantial magnitude.

Two significant deductions may be drawn from the data as a whole. First, appropriate preparation of protein hydrolysates may offer a means of extracting unavailable or indigestible protein from sources not recognized as suitable food products. Second, the mere stipulation of a given percentage of protein in foods or feed stuffs is inadequate in describing their protein value. For instance, in using the nitrogen balance data as a basis of interpretation the soy protein has only about 50 per cent the value of the lactalbumin and about 60 per cent the value of casein; the other data are in substantial agreement. From the standpoint of protein conservation and food economy it is seemingly apparent that as a particular protein more nearly approaches the ideal a commensurate reduction in the amount of dietary protein could be made. The physiological aspects of the lower protein intake may also be of importance from the standpoint of health because the data indicate that a large proportion of the nitrogen absorbed from inferior protein is excreted without commensurately contributing to physiological functions.

The average survival period of the groups of animals used in these investigations when furnished the nitrogen-free diet following the test period tends to indicate a significant effect of the previous protein intake. The average survival time for the lactalbumin group was about 30 per cent longer than for the soy protein group, and the survival time for the casein group was about 24 per cent longer than for the soy protein group.

One may presume that the variations observed are due to an imbalance or deficiency of amino acids, or possibly to an absence of physiologically important linkages in the inferior protein molecule. If evidence should clearly disclose that the former condition is the cause of the differences, the fortification and enhancement of a deficient protein by appropriate synthetic amino acids in proper balance should prove to be of importance from the standpoint of health and economy and conservation of natural food protein.

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STUDIES OF THE EFFECT OF HEAT ON MILK DIALYSATES

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About twenty years ago Grindrod (3) patented a process for dialyzing unsterilized, evaporated milk, and claimed that the removal thereby of 10 per cent of the salts resulted in a greatly improved flavor and color. He indicated that the calcium, citrate, and phosphate ions in excess were detrimental to flavor and color, although his evidence to prove this point was not conclusive. Kass and Palmer (5) showed that citrates and phosphates catalyze the color development or caramelization reaction but they did not study flavor. The claims of Grindrod for flavor improvement prompted us to investigate further the relation of dialysis to heated flavor development.

In checking Grindrod's claims it was found that dialyzing forewarmed or evaporated milk did result in a decreased flavor when the milk was sterilized. However, when the milk was reconstituted with respect to eight known constituents, for which analysis had been made, no improvement in flavor resulted when the milk was sterilized. When raw milk was dialyzed and then forewarmed and evaporated an improved flavor resulted even though the milk had been reconstituted with respect to the known eight constituents. These results indicated that unheated milk contains an unidentified substance which is dialyzable and which contributes to the flavor development of sterilized evaporated milk. These results also indicate that if the milk has been heated before dialysis this substance is not removed by dialysis. In the latter case, however, the salt balance of the milk must be restored before the unknown substance will develop its flavor upon sterilization.

Aging experiments on these dialyzed (reconstituted) samples demonstrated that any flavor improvement was temporary in effect. In fact dialyzed evaporated milk became tallowy in storage whereas milk dialyzed prior to any heat treatment showed about the same degree of aged flavor as the control. The commercial impracticality of using such a procedure for improving the flavor, and the fact that only temporary effects were gained, were, however, not sufficient reasons to justify abandoning the approach of dialysis studies toward learning more about the cause and source of heated milk flavor and aroma.

The dialysis experiments demonstrated that some unrecognized substance of low molecular weight was being removed from milk by dialysis which contributed to the flavor of heated milk. It was discovered that the dialysate from raw milk when heated to 80°–100° C. for 10 minutes did produce a

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cooked milk flavor and odor, though not the typical sulphide aroma from heat-coagulated proteins. This was an unexpected finding and, to our knowledge, has not been reported in the literature dealing with cooked milk flavor. (For a review of the literature on cooked milk flavor the reader is referred to the work of Gould and Sommer (2).) Therefore an extensive effort was made to examine the milk dialysate to determine the source of the cooked flavor and aroma.

EXPERIMENTAL RESULTS

Preliminary fractionation attempts. Initial attempts were made to evaporate the dialysate to dryness under vacuum and at low temperature. It was hoped the residue could then be fractionated through the use of various solvents. This procedure was soon abandoned because of the large volumes of dialysate involved and because the high concentration of lactose and salts in the residue made fractionation difficult. Selective precipitation from the dialysate seemed to be a more reasonable approach.

The addition of $BaCl_2$ to the dialysate from raw milk, following removal of the phosphates at pH 8.5 with $CaCl_2$, forms a precipitate which, upon removal, prevents any cooked odor from appearing in the supernatant liquid upon heating. The barium precipitate upon analysis was shown to consist of barium sulphate and the barium salt of some organic compound. When the organic portion of the precipitate was dissolved and heated, it had a fatty odor but not that of heated milk. Apparently the compound had been destroyed in isolation.

A series of fourteen chemical reagents (mostly salts of heavy metals) of varied properties were prepared in M/1 concentrations for the purpose of attempting to precipitate the cooked flavor precursor from the dialysate without destroying it chemically. Of this whole series it was found that only $HgCl_2$ at pH 8.0 was effective in removing the substance, and fortunately upon regeneration of the precipitate with H_2S and heating the eluate, the cooked aroma was obtained. Considerable experimentation with various salts at varying pH was carried out for the purpose of removing impurities prior to the mercury precipitation. Too high alkalinity (above pH 8.5) was definitely detrimental but it appeared that high acidity in dilute aqueous solution was not harmful even after a week at room temperature.

A working procedure for gross isolation was adopted whereby fresh raw whole milk was dialyzed in cellophane casings against an equal volume of water for 24 hours in the refrigerator. The dialysate was warmed to room temperature and 30 ml. of M/1 $CaCl_2$ were added per liter of dialysate. The pH was adjusted to 8.0 to remove the phosphates, which settled from the supernatant liquid permitting decantation and centrifuging. 100 ml. of a 0.2 M $HgCl_2$ solution were added per liter of supernatant liquid and the pH was adjusted to 7.8 to 8.0. A heavy yellow precipitate settled rapidly and was filtered. This precipitate was washed from the filter paper and suspended in a minimum of water. H_2S was bubbled into the solution while being shaken mechanically for about 30 minutes. The black mercury sulphide was filtered off and H_2S was removed by evacuation and slight evaporation of the solution. The pH of this Hg-free solution was approximately 2.0 probably due to the HCl present from $HgCl_2$ used for precipitation. This solution has always given the cooked aroma in repeated isolation experiments.

Many fractionation experiments and analyses have been carried out using this Hg-free solution as the starting material. A preliminary analysis of this Hg eluate gave the results shown in tables 1 and 2.

TABLE 1

Quantitative analysis of Hg eluate; 100 ml. from 5 gallons of dialysate

Constituent	Concentration	
	mg. per cent	*per cent of solids*
1. Total solids	420.0	100.0
2. Total nitrogen	55.0	13.1
3. Amino nitrogen	11.0	2.62
4. Reducing material calculated as glucose	26.5	6.3
5. Phosphorus	0.0	0.0
6. Sulphur	0.0	0.0
7. Arginine	3.4	0.8
8. Tyrosine	4.2	1.0
9. Creatine and creatinine	17.6	4.2

TABLE 2

Qualitative tests on Hg eluate

Chemical Test	Result
1. Pentose	Questionable
2. Tryptophane	Present
3. Histidine	Present
4. Aldehyde (with fuchsin-sulphurous acid)	Absent
5. Acetyl-glucosamine	Absent
6. Na-nitroprusside and KCN	Absent
7. Labile S with hot alk. $PbAc_2$	Absent
8. Biuret	Absent
9. Phenylhydrazine ppt.	Absent
10. Semicarbazone ppt.	Absent

It is concluded that the solution represents a mixture of substances highly nitrogenous in character and having a high reducing power. Some of the reducing power probably is due to contaminating lactose. The absence of P and S is highly significant and limits the field of suspected compounds considerably.

Since the Hg eluate undoubtedly represented a mixture of substances, various means of fractionation were tried. $PbAc_2$ was found to remove a large proportion of the nitrogenous and highly reducing materials which did not show cooked aroma upon regeneration with H_2S and heating. A white crystalline fraction characterized by hexagonal plates was obtained from this Pb eluate. The total amount of cooked aroma substance seemed to decrease with the Pb treatment and it was finally concluded that a degradation accompanied the treatment. Therefore it was abandoned.

An attempt was made to utilize the synthetic resins Amberlite IR–4 and IR–100 for purifying and separating the cooked flavor producing substance from impurities. Block (1) successfully separated the basic amino acids from protein hydrolysates by use of these resins. It was found that the cooked flavor substance could be absorbed from solution by the IR–100 after a preliminary treatment with IR–4 but no reagent was discovered which would elute the desired material from the resin. The possibility also exists that our substance was destroyed in the treatment thus accounting for the failure to elute.

The most promising method of fractionation of the mercury eluate seemed to be that of evaporating under high vacuum and low temperature, removal of crystalline fractions or precipitates as they formed, and continued evaporation to dryness, testing all precipitates for cooked flavor by solution and heating. The cooked flavor producing substance remains in solution until practically all the H_2O has been evaporated and after removal of two or three yellow crystalline or granular fractions possessing no cooked flavor properties. The final liquid was first thought to be a heavy viscous yellow oil but actually it was a syrup since it was found that it could be dried by continued evacuation with the Hy-Vac pump. It dried on the walls of the flask as a white crystalline material, interspersed with red-yellow streaks. Strong vapors of HCl were present in the flask and accounted for the high acidity of the mercury eluate.

From a solubility basis it was found that the dry crystalline material is most soluble in water, slightly soluble in methyl alcohol, and less soluble in ethyl alcohol. It is insoluble in acetone, dioxane, petroleum, and ethyl ether, chloroform and isobutyl alcohol. A further purification was attempted on the basis of differential solubility.

The highly viscous syrup prior to removal of the last traces of water was diluted with an equal volume of methyl alcohol. A white crystalline fraction separated rapidly which was found to be almost cubical in shape. This fraction dissolved upon the addition of more methyl alcohol. Acetone caused a white sticky precipitate which contracted into a ball. This was centrifuged off and it was found upon dissolving in water and heating that the acetone insoluble portion possessed the cooked flavor.

When the syrup was completely dried and a minimum of methyl alcohol added, a white crystalline fraction similar to the above separated. The addition of more alcohol caused it to dissolve and upon evaporation of this solution it became darker in color, changing from red-yellow to red-black. The dry material was rather brown in color. Resolution in methyl alcohol and evaporation after standing a few hours caused a considerable darkening in color—deep cherry red color. Upon drying it became blacker and changed to a tarry mass. It was noted that acetone after treating with dry HCl had similar properties, changing from yellow-green to red, due to the formation of phorone (4). In this respect our concentrate has a property in common with acetone. A loss of power to produce cooked flavor accompanied the color-changing phenomenon noted above. This loss seemed considerably more rapid in acid methyl alcohol in the absence of water than in the former case where water was used to dissolve the dry material and alcohol used to crystallize. Considerable material was lost in this manner before the conditions for stability were established.

Revised methods of isolation of cooked flavor precursor. Fourteen batches of dialysate varying from 5 to 25 gallons each were prepared during the next period of investigation. In all cases a volume of water was used approximately equal to that of the milk. Skim milk was employed satisfactorily for some of the 25-gallon batches. A tank 8′ × 1′ × 1′ was constructed for large-scale operations, which minimized the labor of filling and tying the cellophane tubes. It was found possible to utilize the dialyzing tubing several times by washing thoroughly and heating in boiling water to destroy bacteria.

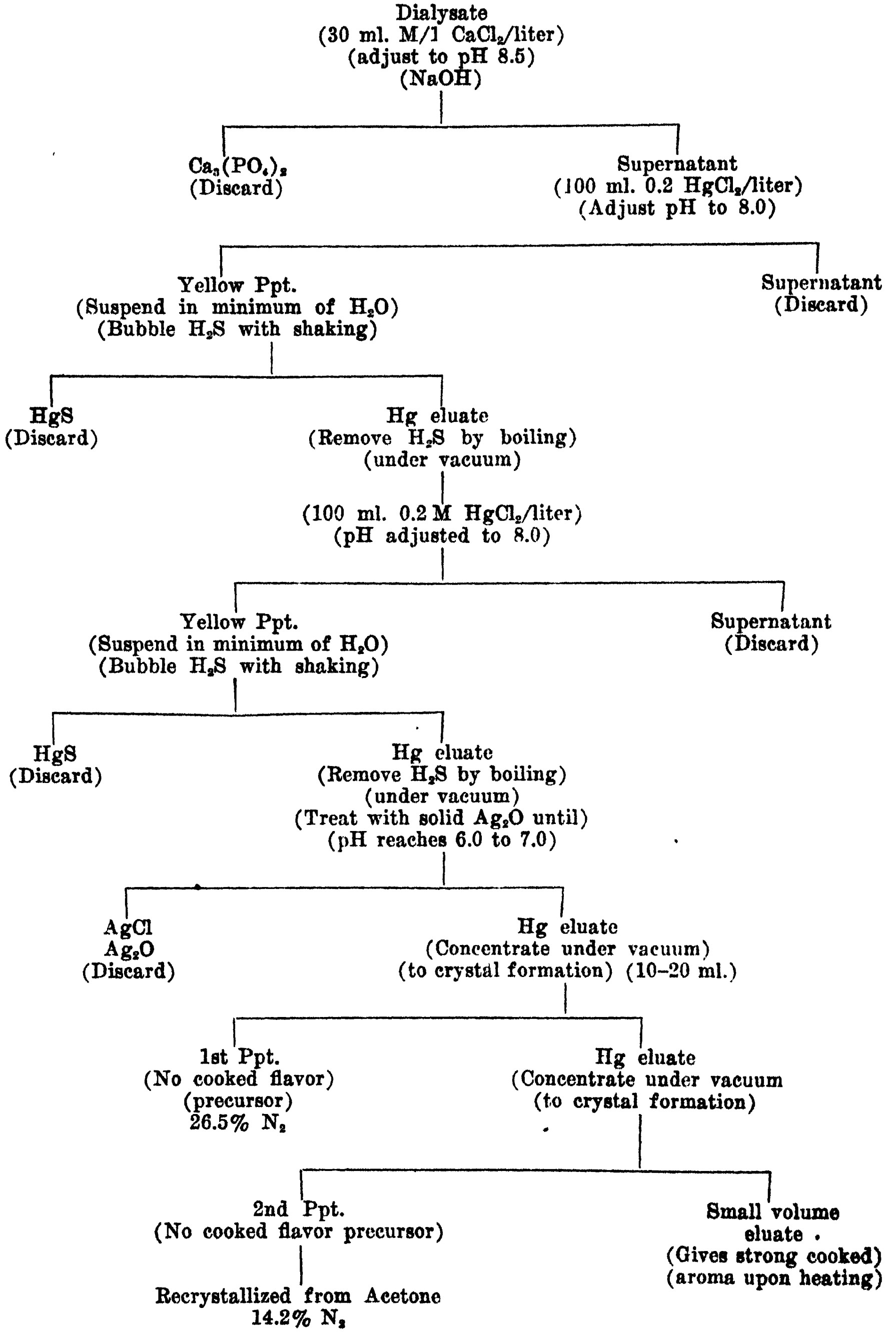
Some additions were made to the isolation procedure. A second mercury precipitation and regeneration was found to be advantageous for removal of lactose. Neutralization of the second mercury eluate with AgO to pH 6.0 to 7.0 followed by filtration of AgCl removed excess HCl which had caused destruction of the cooked flavor precursor, especially when organic solvents were employed for subsequent fractionation.

Various crystalline fractions were isolated from this solution by concentration under vacuum and by means of organic solvents. These fractions were subjected to analyses and recrystallizations with the ultimate hope of identifying them.

Properties of fractions. It was recognized that the substance isolated by the adopted isolation procedure consisted of a mixture of substances as evidenced by variable nitrogen contents and reducing values. Attempts to fractionate this substance resulted in impure fractions some of which were crystalline and possessed varying degrees of cooked aroma precursor as evidenced by heating 10 mg. dissolved in 10 ml. H_2O. The results indicated that the cooked aroma precursor was carried along in these fractions as an impurity and could produce a strong cooked aroma when present in concentrations lower than 1 mg./ml. Later work revealed that 0.2 mg./ml. was sufficient to give a strong cooked aroma and amounts less than 0.2 mg./ml. could be detected.

It was noted that the cooked flavor substance was only slightly soluble in neutral methyl alcohol. Therefore repeated attempts at purification

MILK DIALYZED AGAINST AN EQUAL VOLUME OF WATER



using this organic solvent were tried. Unfortunately an inactivation or destruction appeared to accompany repeated treatment with methyl alcohol. It was reasoned that if a trace of formaldehyde were present in the alcohol it could react with the amino groups in the cooked flavor substance and thus account for the loss of active substance. Even though freshly purified methyl alcohol was used there resulted a destruction of the cooked flavor substance. Therefore fractional crystallization from neutral aqueous medium was resorted to and found to cause less destruction than did the methyl alcohol.

Six lots of dialysate were subjected to this fractional crystallization treatment. A crystalline fraction of rods separated at 10–20-cc. volume (from 5 gal. dialysate) and was filtered off. This fraction (recrystallized) had 26.5 per cent N_2 and did not produce cooked aroma on heating. A strong Schiff test was given for this fraction whereas a negative test was obtained in those fractions showing strong cooked aroma.

Further evaporation of the supernatant liquid caused a needle or rosette fraction to crystallize. This fraction was first thought to be the cooked aroma substance, but when care was taken to remove all the supernatant liquid by suction, it likewise was found to be inactive. The cooked aroma substance still remained in the supernatant liquid. This needle-rosette fraction crystallized from cold 25 per cent acetone solution readily. It was found to contain approximately 14.2 per cent N_2.

The volume of solution remaining after removal of the two crystalline fractions was exceedingly small, thus preventing further fractional crystallization attempts. Further evaporation of the supernatant liquid resulted in a sticky, hygroscopic non-crystalline mass. Larger initial volumes of dialysate will be necessary before this last fraction can be obtained in quantities large enough for analysis.

Identification. Even though the cooked flavor precursor has not been isolated in the pure state attempts have been made to identify the substance in the impure mixture.

It has been concluded that the reducing power found in original samples (table 1) is due to contaminating lactose and not a property of the active substance. In all cases where a highly active substance has been purified a rather high but variable nitrogen content has been found. Two contaminating crystalline fractions have been isolated and recrystallized which have high nitrogen contents. Large amounts of these fractions remaining in the active substance may be misleading us with regard to nitrogen content.

The possibility that the two crystalline fractions might be inactive degradation products of an active parent substance has been considered. It has always appeared that we suffered a continual loss of active substance in our isolation attempts. This loss corresponds to a chemical degradation with time rather than a manipulative loss which might accompany inefficient separation by precipitation or elution. The two crystalline fractions have been heated together at varying proportions of concentration with no cooked aroma resulting, thus eliminating the possibility of an interaction of the two compounds to produce the cooked aroma.

Attempts to identify the cooked aroma substance by heating a few milligrams of various chemicals dissolved in water or 5 per cent lactose solution have failed. To date 46 chemical compounds of known structure, which might be suspected as being the cooked flavor precursor, have been tried but not one has had an aroma similar to that of our unknown substance. Types of compounds tried have been amino acids, vitamins, purine bases, amines and substituted amines and a few miscellaneous compounds. Apparently the final solution of the problem will appear only upon isolation and identification of the particular compound in question.

DISCUSSION

After much of this work had been carried out it became apparent that other methods of procedure would have to be employed to prevent the large degradative losses of active substance. A re-evaluation of the effect of pH on stability showed that acidity was far more destructive than originally believed. Neutral solutions were the most stable, so any new attempts at isolation should be based on this consideration. Possibly a less acid mercury compound such as $HgAc_2$ or the use of a less acid regenerating agent such as Na_2S or $(NH_4)_2S$ could be used satisfactorily.

The cooked flavor precursor may be any one of a multiple of nitrogenous products in milk. A consideration of all the properties and reactions of the cooked flavor precursor as well as the analysis of some of the compounds isolated which might be considered as degradation products suggest the possibility of phospholipids and cerebrosides of milk (6) being the compounds involved. They are in general slightly soluble in water, non-crystalline, should dialyze through cellophane membranes because of their surface activity and their low molecular weight of about 800, they form compounds with $HgCl_2$, are precipitable with acetone and are labile compounds to acid, alkali and heat. The acid regeneration treatment of the mercury precipitate could cleave the molecule such that the phosphorus remained insoluble, but the remaining portion of the molecule could be eluted and still give rise to the cooked aroma upon heating. Of course there is no phosphorus present in cerebrosides so this explanation is not necessary for this compound. They all contain nitrogen residues and fatty acids; cerebrosides contain the carbohydrate galactose, a reducing sugar which we could have erroneously thought to be lactose.

The needle or rosette fraction with a nitrogen content of 14.2 per cent agrees fairly well with either trimethylamine hydrochloride or amino ethanol hydrochloride (theoretical N for both = 14.7 per cent). The rod-shaped fraction showing a nitrogen content of 26.5 per cent might be ammonium chloride (theoretical N = 26.2 per cent) except that its solubility in water is far too low.

SUMMARY AND CONCLUSIONS

Dialysis experiments on raw milk demonstrated that some unrecognized substance of low molecular weight could be removed, which contributed to the flavor of heated milk.

It was discovered that the dialysate from raw milk when heated to 80°–100° C. produced a heated milk flavor and odor, though not the typical sulphide aroma obtained from heat-coagulated proteins. The flavor and aroma of heated milk appears to be derived from two sources—the heat coagulable proteins and a dialysate factor.

The addition of a barium salt prevented the production of a cooked aroma upon heating. Because it was not found possible to regenerate the cooked aroma precursor from the barium precipitate, it was concluded that the desired compound had been destroyed.

Of fourteen other precipitating agents tried, only $HgCl_2$ at pH 8.0 was effective in removing the cooked flavor precursor from the dialysate. Cooked aroma was obtained by heating the eluate from the H_2S regenerated precipitate after complete removal of residual H_2S.

Some of the properties of the partially purified cooked flavor precursor were established, significant of which are its solubility in water, lesser solubility in methyl alcohol, slight solubility in ethyl alcohol and insolubility in other solvents such as acetone, ethers and chloroform. There is no P or S present in the compound. It is unstable in aqueous alkaline solutions, but somewhat stable in aqueous acid solution. It is very unstable in acid methyl alcohol solution free from water, showing a reaction similar to that of acetone in the presence of dry HCl.

Fourteen batches of dialysate varying from 5 to 25 gallons each were used for the purification of the cooked aroma precursor by revised methods. A second mercury precipitation and the use of Ag_2O to remove excess HCl improved the isolation procedure.

Two crystalline fractions have been isolated and recrystallized from the purified cooked flavor concentrate, neither of which is the desired cooked flavor precursor. A rod fraction having 26.5 per cent nitrogen and a needle-rosette fraction with 14.2 per cent nitrogen have been obtained in good quantity and recrystallized with the ultimate hope of identifying them.

The cooked flavor substance has not been isolated in its pure crystalline state. Slow inactivation or destruction with all techniques tried to date has made the problem difficult.

Of 46 chemical compounds of known structure which might be suspected as the cooked flavor precursor, not one produced an odor resembling that of the unknown substance when heated in aqueous solution.

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MODIFICATION OF THE PHOSPHATASE TEST AS APPLIED TO CHEDDAR CHEESE AND APPLICATION OF THE TEST TO FLUID MILK

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A description of a phosphatase test applicable to Cheddar cheese, to determine whether or not the milk used in making the cheese had been pasteurized, was published by us in 1945 (1, 2). Recently, as a result of additional research on the chemistry of (a) the enzymic hydrolysis and of (b) the indophenol color reaction, the test has been improved further to make the results more precise and quantitative. This report is a description of the modified test applied to Cheddar cheese and also to fluid milk. The modifications are described briefly as follows.

(a) The optimal pH of hydrolysis for the production of phenol in the presence of barium buffer substrate, with the concentration of buffer used in this test on milk, has been found to be 10.0 to 10.05, rather than a lower value found previously with a buffer substrate containing a relatively high concentration of sodium tetraborate. The composition of the buffer has been adjusted accordingly, and it yields relatively uniform pH values with different samples. The increased precision of the pH adjustment with the buffer described herein has resulted in an increase in the quantity of phenol liberated in definitely positive tests over that liberated in the test described earlier, and the additional advantage in the use of barium in the test, to reduce the effects of phosphates and of other interferences, has been verified.

(b) Results obtained in research on the indophenol color reaction have shown that the use of an auxiliary protein precipitant makes it possible to increase the sensitivity, precision, and quantitative accuracy of the test. With an auxiliary precipitant, the need for boiling the test after incubation has been eliminated—the test is heated only to 90° C. (194° F.), sufficiently to inactivate the enzyme. The elimination of boiling has been found advantageous since boiling causes some hydrolysis of the disodium phenyl phosphate and thus results in the formation of a trace of blue color in the blank. Moreover, prolonged boiling may result in some caramelization. Thus the use of an auxiliary protein precipitant results in complete protein precipitation without boiling, yielding a perfectly clear, colorless filtrate..

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Numerous protein precipitants were investigated, and it was found that BQC produces considerably more blue color, with excess phenol, in the presence of zinc than in the presence of lead, and that under such conditions copper has a remarkable catalytic effect on the rate of formation and the final amount of blue indophenol color produced and also aids in reducing the amount of visible yellow color in the blank. Therefore, a combination of zinc and copper salts was introduced as an auxiliary precipitant in the modified test.

Coincident with the demonstration of the value of copper in the development of blue color in the test, it became necessary to determine the quantity of copper to use in testing different products. A difficulty in the use of copper is the fact that an excess quantity of it imparts a copper-blue color to the filtrate. This difficulty was overcome by determining the optimal quantity of copper that would produce the desired catalytic effect without causing appreciable interference. The required quantity of the copper salt—different for milk than for cheese—was then combined with a quantity of zinc salt sufficient to complete the precipitation of the proteins and reduce the pH to 9.1 to 9.2.

The modification includes also the development of an additional buffer, made with sodium metaborate and sodium chloride and referred to as the color-development buffer, added to the test filtrate to automatically adjust the pH correctly. The optimal pH for the complete BQC-phenol coupling reaction in the improved test has been found to be within a range of 9.1 to 9.5. More yellow off-color is produced at still higher pH values, and slightly more blue and less yellow at pH 9.1. However, the reaction is slower at pH 9.1 than at 9.5. This improved color-development buffer adjusts the pH at 9.3 to 9.4—suitable for complete color development in the time specified, with a minimum of off-color. The use of it performs the additional function of diluting the interfering substances present, thus further reducing the reading of the blank.

One of the aims of the development work has been to simplify the phosphatase test. For example, preliminary preparation of the sample with the use of a mortar and pestle or with a blender has not been found necessary; the alkalinity and the strength of the buffers have been adjusted to give results that are reliable for all samples without the need of determining and adjusting the pH when testing; and all reagents are prepared by weighing, hence the need for primary standards or for making solutions of designated normalities for the test has been eliminated.

I. APPLICATION TO CHEDDAR CHEESE

Reagents

Barium borate-hydroxide buffer for Cheddar cheese. Dissolve 25.0 g. of C.P. barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$, fresh, not old) in distilled water and dilute to 500 ml. Dissolve, in another flask or cylinder, 11.0 g. of C.P. boric acid (H_3BO_3) and dilute likewise to 500 ml. Warm each to 50° C. (122° F.), mix the two solutions together, stir, cool to approximately 20° C. (68° F.), filter, and stopper the filtrate tightly. The pH of the barium borate-hydroxide filtrate is approximately 10.6, and a mixture of 9 ml. of it with 0.5 g. of Cheddar cheese yields, with different samples, pH values of 10 to 10.05 ± 0.15.

Disodium phenyl phosphate substrate. Specify phenol-free, crystalline disodium phenyl phosphate.[1] Prepare a stock solution by dissolving 1.0 g. in 9.0 ml. of the color-development buffer described below. Before use, remove any free phenol by adding 1 drop of BQC, developing the color for 30 minutes at room temperature or 15 minutes at 37–38° C. (99–100° F.) and extracting the color with 5 ml. of n-butyl alcohol (Scharer extraction method). Draw off and discard the alcohol. The extraction should be repeated if there is much blue color in the alcohol layer, but usually one extraction is sufficient. This stock solution should be kept in a refrigerator and, if used again after standing for several days, should be re-extracted before use.

To prepare the buffer substrate for several days' use, make up this entire stock solution of purified disodium phenyl phosphate to 1 liter with the barium buffer; or for daily use, prepare fresh buffer substrate by adding 1 ml. of this stock solution of purified disodium phenyl phosphate to 100 ml. of the barium buffer. Store the buffer substrate in a refrigerator.

It has been found that 1 g. of disodium phenyl phosphate per liter, as described above, is sufficient for detecting under-pasteurization. However, for quantitative tests on raw or definitely under-pasteurized samples, somewhat more phenol is liberated if the concentration of purified disodium phenyl phosphate is increased to 2 g. per liter or to 2 ml. of the stock solution per 100 ml. of the buffer.

Zinc (or zinc-copper) precipitant for Cheddar cheese. Weigh 6.0 g. of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and make up to 100 ml. with distilled water. For testing non-ripened cheese, not more than a few days old, for use in the rapid field test, and for use in all tests in which the butyl alcohol extraction method is used, add 0.1 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$) before making up with water.

The optional use of either zinc or zinc and copper is suggested for the test on cheese, since it has been found that, in tests on ripened cheese, copper catalyzes also the formation of a violet- or purple-colored compound resulting from chemical reaction of BQC with decomposition products (probably amino acids) present in ripened cheese. In tests that are to be read in aqueous solution, the use of the zinc-copper precipitant is suggested for testing green (non-ripened) cheese—not more than a few days old—and the use of the zinc precipitant without copper is suggested for testing ripened cheese.

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[1] This compound, relatively pure, is obtainable from Applied Research Institute, 139 Fifth Avenue, New York 10, New York.

The violet-colored compound produced in the presence of copper in tests on ripened cheese is not sufficiently soluble in butyl alcohol to cause appreciable interference in tests in which the butyl alcohol extraction method is used. Therefore, the use of the zinc-copper precipitant is suggested for all tests in which the color is to be extracted.

Color-development buffer. Mix 6.0 g. of sodium metaborate ($NaBO_2$)[2] with 20.0 g. of sodium chloride and make up to 1 liter with distilled water. The pH of this buffer is approximately 9.8, and that of a mixture of 5 ml. of it with 5 ml. of the test filtrate is 9.3 to 9.4.

The color-development buffer is used also for adjusting the pH in the following steps: extracting free phenol from the disodium phenyl phosphate; adjusting correctly the alkalinity of the butyl alcohol; and preparing the phenol standards. Also, a solution containing 1 part of it in 9 parts of water is used for preparing dilutions of the test, in order to reduce the color of strongly positive tests so that they will be in a readable range for colorimetric analyses.

2,6-dibromoquinonechloroimine (BQC) solution (Gibbs' reagent). Dissolve 40 mg. of the pure substance in 10 ml. of methyl or ethyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

To prepare the chloroimine solution from INDO-PHAX tablets,[3] dissolve 1 tablet in 5 ml. of methyl or ethyl alcohol and keep in a dark bottle as indicated above.

The 2,6-dichloroquinonechloroimine reagent (CQC) can be substituted for the dibromo reagent, if desired, provided the standards are prepared with it also, but it requires a slightly longer time for full color development. It, as well as the tablets mentioned above, produces blue color at a slower rate than BQC, but a given quantity of it eventually produces more blue color in strongly positive tests than the same quantity of BQC.

It is recommended that CQC, rather than BQC, be used in the field test, because CQC is relatively stable and is affected less by protein interference in the test.

Butyl alcohol. Specify n-butyl alcohol, B.P. 116–118° C., for making standards and for quantitative work. The cheaper grade of n-butyl alcohol can be used for routine testing. To adjust the pH, mix 50 ml. of the color-development buffer with 1 liter of the butyl alcohol.

Phenol standards. Weigh exactly 1.0 g. of U.S.P. phenol and make up to about 700 ml. with distilled water in a liter flask. Add 150 ml. of the color-development buffer to form a solution of sodium phenolate with a pH of approximately 9.25, which is more stable than phenol. Add 3 ml. of chloroform and water to the 1,000-ml. graduation, and mix. One ml. of this stock solution contains 1 mg. (0.001 g.) of phenol. Pipet 1.0 ml. of the stock solution into a liter flask, add water to the 1,000-ml. graduation, and mix. One ml. of this standard solution contains 1 mmg. (0.000001 g.—1 microgram, gamma, or unit) of phenol. Prepare additional solutions as needed, containing, for example, 2, 5, 10, 20, and 40 mmg. of phenol per ml. by diluting 2.0 ml. of the stock solution to 1,000, 5.0 to 1,000, 5.0 to 500, 10.0 to 500, and 20.0 to 500 ml., respectively. From these, measure appropriate quantities into a series of tubes, preferably graduated at 10.0 ml., to provide a suitable range of phenol standards containing 0 (blank) 0.5, 1.0, etc., to 40 units.

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[2] Obtainable from Amend Drug and Chemical Company, 117 East 24th Street, New York 10, New York.

[3] Obtainable from Applied Research Institute.

A solution containing copper is added at this point to increase the brightness of the blue color and to improve the stability of the standards. Prepare a 1 per cent solution of copper sulfate in distilled water, dilute 5 ml. of it to 100 ml., and pipet 1 ml. of this 0.05 per cent solution into each tube.

Add color-development buffer to each tube to bring the volume to 10.0 ml. Add 4 drops (0.08 ml.) of BQC to each, and mix. Allow to develop for at least 30 minutes at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards at this point with butyl alcohol as described in the laboratory test.

Read the color intensities with a photometer with the proper filter, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve; or, for use in visual comparisons, store the standards in a refrigerator.

SAMPLING

Take a sample from the interior of the cheese with a *clean* Roquefort trier, place it in a small tube, stopper the tube, and keep it in a refrigerator.

LABORATORY TEST

Weigh carefully, on a *clean* watch glass, 0.5 g. of cheese and place it in a culture tube 16 or 18 × 150 mm. Macerate the sample with a glass rod about 8 × 180 mm., pipet in 1.0 ml. of buffer substrate, complete the maceration, pipet in 8.0 ml. more of buffer substrate (total 9.0 ml.), and mix thoroughly with the rod. To include in the test the fatty material adhering to the rod, cut a piece of filter paper approximately 1 × 1¼″, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube in such a manner as to wipe the rod clean, and insert the paper with the fat into the test. Stopper the tube and mix the contents thoroughly by shaking. Incubate in a water bath at 37–38° C. for 1 hour, preferably shaking the tube occasionally. Place the tube in a beaker of boiling water and leave it for 1 minute, or heat the contents to at least 90° C. (194° F.). Cool to room temperature. Pipet in 1.0 ml. of the zinc precipitant (for cured cheese) or the zinc-copper precipitant (for green cheese), mix thoroughly, and filter (5-cm. funnel, 9-cm. Whatman No. 42 paper recommended). Pipet into another tube (preferably graduated at 5.0 and at 10.0 ml.) 5.0 ml. of the filtrate and add 5.0 ml. of color-development buffer. Add 4 drops of BQC, mix, and let stand at room temperature for 30 minutes or at 37–38° C. for 15 minutes.

At this point, the blue color in tests with phosphatase values of about 5 or more units can be detected visually in the aqueous solution.

For detecting under-pasteurization in border-line instances—*e.g.*, tests yielding 0.5 to 5 units of color—and for more quantitative results with all samples, the intensity of the blue color is increased and that of the off-color in the blank is diminished by extracting the color with n-butyl alcohol. Add 5.0 ml. of the alcohol (Scharer extraction method) and invert the tube slowly several times. Compare visually the blue color in the alcohol layer with the colors of standards, or draw off and filter the alcohol and measure the color in it by means of a colorimetric instrument.

A control (blank) determination should be conducted with every sample tested, using 0.5-g. of cheese, macerating it in the tube and heating it to at least 90° C. before adding the buffer substrate. Subtract the value of the blank from that of each test.

Alternatively, to avoid gumminess of the blank when it is heated, macerate it in the tube, pipet in 1.0 ml. of the barium buffer (without substrate added), then mix and heat to 90° C., cool, pipet in 8.0 ml. of the barium buffer substrate, and proceed with incubation and subsequent steps as described above.

Since 0.5 g. of cheese is used and the quantity of filtrate used is equal to one-half the total quantity of solution (buffer substrate plus precipitant), the result obtained as described above is recorded as units of color or phenol equivalents in mmg. per 0.25 g. of cheese. However, the result may, if desired, be converted to phenol equivalents in mmg. per 1 g. of cheese.

If it is more convenient to add a total of 10.0 rather than 9.0 ml. of buffer substrate, this may be done and it will not affect significantly the pH of the mixture. The additional dilution of the test will increase slightly the quantity of phenol liberated. In calculating the result obtained when using 11.0 ml. of liquid in the mixture and 5.0 ml. of filtrate, multiply the value obtained by 1.1 to obtain the result in phenol equivalents per 0.25 g. of cheese.

In testing samples that are observed during color development to be strongly positive—*e.g.*, approximately 20 units or more—in which the quantity of BQC specified is not sufficient to combine with all of the phenol, quantitative results may be obtained by means of dilutions made as follows: While the color is developing, pipet out exactly half the contents (5.0 ml.) into another tube, pipet in with it 5.0 ml. of a 1 to 9 dilution of the color-development buffer, add 2 drops more of BQC, and allow to develop. To reduce the color at the end of the development period to conform with the range of standards or of the photometer, repeat this one-half dilution procedure as many times as necessary, allowing the full specified time for color development after the last addition of BQC. With each test diluted thus, conduct a blank determination in the corresponding manner.

Samples of cheese made from raw milk may yield values as high as 1,000 units of color per 0.25 g. of cheese. Therefore, to save time when testing such cheese, the dilutions may be increased. For example, a first dilution of one-fifth (2.0 ml. of test solution plus 8.0 ml. of diluted color-development buffer) rather than one-half may be used, followed by a second dilution of one-fifth (2.0 ml. of the diluted test solution plus 8.0 ml. of the diluted buffer), thus diluting the solution one-twenty-fifth in only two steps. Develop the color as indicated above.

Photometric Determination

To read the color in aqueous solution, use a filter with maximum light transmission in the region of 610 mμ wavelength.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 minutes to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ¼ inch thick from a rubber stopper of suitable diameter to fit in the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section in the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wavelength.

If more than approximately 4 ml. of butyl alcohol is required for the photometer used, the test is conducted in a larger tube and the color is extracted with the necessary quantity of butyl alcohol rather than with 5 ml. specified above.

Rapid Field Test

Macerate 0.5 g. of cheese with the buffer substrate, as described in the laboratory test, adding a total of 9 ml. of buffer substrate. Mix thoroughly and incubate for 30 minutes at 37–38° C. Add 1.0 ml. of the zinc-copper precipitant. Add 8 drops of CQC, mix thoroughly, and allow the color to develop for 15 minutes at room temperature. Conduct a control (blank) determination using a 0.5-g. sample of the cheese, macerating it in the tube and heating it to at least 90° C. before adding the buffer substrate.

To increase the precision, especially for border-line tests, the color may be extracted with 5 ml. of the n-butyl alcohol before making comparisons with the colors of standards. When it is necessary only to detect under-pasteurization, a few standards, *e.g.*, 1, 2, 5, and 10 units, may be sufficient; under these conditions, extraction with butyl alcohol is necessary for only those tests in which the results are doubtful.

Precautions

The presence of free phenol in the buffer substrate is frequently the cause of a trace of blue color in the blank determination. It is therefore desirable to purify the stock solution of disodium phenyl phosphate by extracting it immediately before use.

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware and stoppers should be scrupulously clean and it is desirable to soak them in hot, running water after cleansing.

The solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

Interpretation

Results obtained with this test on cheese made from milks containing small proportions of raw milk in pasteurized milk, and also corresponding results published earlier, show that the enzyme is more concentrated in cheese than in the milk from which it was made. The earlier results indicate also that the enzyme is not completely inactivated under the temperature and time conditions specified in pasteurization standards. The modified test described here yields less blue color in the blank and also less yellow off-color in the blank than earlier tests, and therefore yields results that are more precise for detecting under-pasteurization. On the basis of our results, it is suggested tentatively that values greater than 3 mmg. of phenol per 0.25 g., or 12 mmg. per 1 g., of cheese be considered as indicating under-pasteurization.

Results

Results obtained in using the modified test on samples of cheese of different ages show that the enzyme is much more stable in cheese than has been generally supposed. Data obtained on raw-milk cheese 18 months old, illustrated in table 1, show phosphatase values more than half as great as those obtained in tests on raw-milk cheese 2 days old. Moreover, analyses made with definite quantities of phenol added to test filtrates prepared with samples of cheese of various ages, without disodium phenyl phosphate substrate added, show that the color-reaction inhibition or interference increases with the age of the cheese, and that the increased interference is the result of decomposition products accumulating in the cheese as it ripens. As the interference (less blue and more yellow color in the test) increases with the age of the cheese, the amount of off-color in the blank increases, the efficiency of the BQC reagent in the test diminishes, and the results for detecting under-pasteurization become slightly less precise. Finally, phenol present in the cheese-test filtrate cannot be determined quantitatively regardless of how much BQC is added. The failure to determine the phenol quantitatively becomes more and more marked as the age of the cheese increases.

TABLE 1

Phosphatase values of Cheddar cheese of different ages and of the milk and whey

Lot No.	Age of cheese when tested	Description of milk	Phosphatase value, phenol equivalent		
			Cheese	Milk	Whey
			mmg./g.	*mmg./ml.*	*mmg./ml.*
1031	18 months	Raw	1640		
1107	18 months	Raw	1960		
514–1	2 days	Raw	3200	2060	920
514–2	2 days	2.5% raw milk in past. milk	134.4	56.6	23.2

The earlier results demonstrated that the enzyme present in milk is concentrated in the cheese at the expense of the whey. This fact has been verified in the present work with the modified test, as indicated by illustrative results shown in table 1. The values obtained on samples of cheese made from milk containing small proportions of raw in pasteurized milk are generally two to three times as large, per unit of volume or of weight, in the cheese as in the milk, and considerably smaller in the whey than in the milk.

II. APPLICATION TO FLUID MILK

Reagents

Barium borate-hydroxide buffer substrate for milk. Dilute a definite quantity of the barium buffer described under the cheese test with an equal quantity of distilled water. A mixture of 8 ml. of this diluted buffer with 1 ml. of milk yields, with different samples, pH values of 10.0 to 10.05 ± 0.15. Prepare and purify the disodium phenyl phosphate substrate and add it to the diluted buffer in the manner described under the cheese test.

This buffer substrate for milk is suitable also for testing cream and whey.

Zinc-copper precipitant for milk. Mix 3.0 g. of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with 0.6 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$) and make up to 100 ml. with distilled water.

Other reagents. The color-development buffer, the BQC solution for use in the laboratory test, the CQC solution for the rapid field test, the butyl alcohol, and the phenol standards are prepared in the manner described under the cheese test—except, however, that 2 drops of BQC is sufficient for the low-phenol or border-line standards.

Laboratory Test.

Mix the sample of milk well and pipet 1.0 ml. into a tube 16 or 18 × 150 mm., preferably graduated at 5 and at 10 ml. Pipet in 8.0 ml. of the buffer substrate and mix the contents thoroughly by shaking. Incubate in a water bath at 37–38° C. (99–100° F.) for 1 hour, preferably shaking the tube occasionally. Place the tube in a beaker of boiling water and leave it for 1 minute, or heat the contents to at least 90° C. (194° F.). Cool to room temperature. Pipet in 1.0 ml. of the zinc-copper precipitant for milk, mix thoroughly, and filter as described under the cheese test. Pipet into another tube 5.0 ml. of the filtrate and pipet in 5.0 ml. of color-development buffer. Add 2 drops (0.04 ml.) of BQC, mix, and let stand at room temperature for 30 minutes or at 37–38° C. for 15 minutes.

In making quantitative tests on samples that are observed during color development to be strongly positive, use 4 drops of BQC and prepare color dilutions by the method described under the cheese test.

In the test as modified for milk, a quantity of phenol as small as 1 mmg. can be detected visually and extraction with butyl alcohol is not necessary. If it is desired to use the butyl alcohol extraction method for a still greater increase in sensitivity, perform the extraction as described under the cheese test.

A blank determination should be conducted every time a new lot of buffer substrate—or of any of the other reagents—is used, using 1.0 ml. of milk that has been heated to at least 90° C. before adding the buffer substrate. Subtract the value of the blank from that of each test.

Since 1 ml. of milk is used and the quantity of filtrate used is equal to one-half the total quantity of liquid, the result obtained as described above is recorded as units of color or phenol equivalents in mmg. per 0.5 ml. of milk. However, the result may, if desired, be converted to phenol equivalents in mmg. per 1 ml. of milk.

If it is more convenient to add a total of 10.0 ml. rather than 8.0 ml. of buffer substrate, yielding a total of 12.0 ml.in the test mixture, this may be done and it will not affect significantly the pH of the mixture. In calculating the result obtained when using 12.0 ml. of liquid in the mixture and 5.0 ml. of filtrate, multiply the value obtained by 1.2 to obtain the result in phenol equivalents per 0.5 ml. of milk.

When using either phenol standards or a photometer, make the readings as described under the cheese test.

Rapid Field Test

Mix the sample of milk well, pipet 1.0 ml. into a tube, pipet in 8.0 ml. of buffer substrate for milk, and mix thoroughly, as described under the laboratory test for milk. Incubate for 30 minutes at 37–38° C. Pipet in 1.0 ml. of the zinc-copper precipitant for milk. Add 6 drops of CQC, mix well, allow the color to develop for 15 minutes at room temperature, and complete the determination as described under the rapid field test for cheese.

Precautions

The same precautions apply in the milk test as described under the cheese test.

Interpretation

On the basis of our results, it is suggested tentatively that values greater than 2 mmg. of phenol per 0.5 ml., or 4 mmg. per 1 ml., of milk be considered as indicating under-pasteurization.

Results

In the experiments on cheese it was found that barium reduces the inhibition of enzymic hydrolysis caused by the presence of phosphates and some other anions. These results have been verified in tests on milk, wherein the use of a barium borate substrate yielded an average of approximately 12 per cent more hydrolysis than did a sodium tetraborate substrate under comparable conditions.

Spectrophtometric data obtained on milk-test filtrates are shown in figure 1.

The curves indicate the increase in intensity of the blue color at wavelengths in the region of 610 mμ and the decrease in intensity of the interfering color in the region of 450 mμ, when 0.04 ml. (2 drops) of BQC is used with the zinc-copper reagent instead of with the lead reagent. With the use of the greater quantity of BQC, the interfering color is increased greatly, especially when the lead reagent is used. This interferes with visual detection of a blue color of low intensity and increases the photometric reading of the blank in the region of 610 mμ wavelength.

The improvement produced with the zinc-copper reagent has made it possible to reduce the quantity of BQC from 4 to 2 drops in the test on milk. Thus the difference between a control test and a test of 1 unit of blue color can be clearly detected visually, making the extraction of the color with butyl alcohol unnecessary.

It was found in this work, and also in experiments conducted with Horwitz (3), that the results of the test are a straight-line function of the concentration of the enzyme present, provided that a sufficient excess of disodium phenyl phosphate is present. However, the concentration of disodium phenyl phosphate specified for detecting under-pasteurization is not suffi-

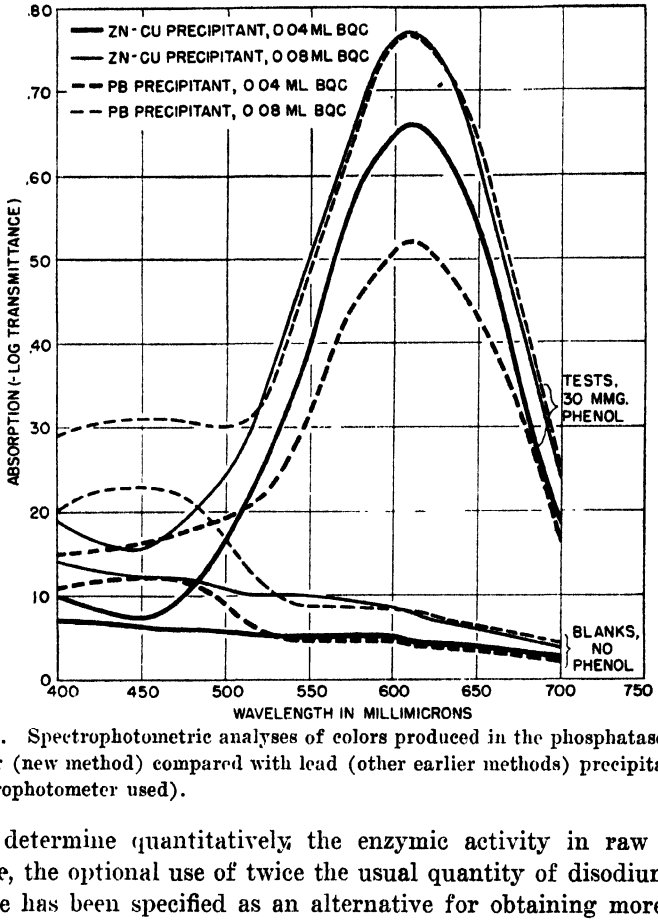

FIG. 1. Spectrophotometric analyses of colors produced in the phosphatase test with zinc-copper (new method) compared with lead (other earlier methods) precipitant (Beckman spectrophotometer used).

cient to determine quantitatively the enzymic activity in raw samples. Therefore, the optional use of twice the usual quantity of disodium phenyl phosphate has been specified as an alternative for obtaining more quantitative results on raw samples.

Tests to determine the precision of the modified method were conducted on standardized 4 per cent milk, on the cream separated from it and stand-

TABLE 2

Phosphatase values of standardized milk and of the corresponding skim milk and standardized cream, with various proportions of raw added to the boiled product (averages for four sets of samples)

Proportion of raw added to boiled product	Phosphatase value, phenol equivalent		
	Whole milk (4.0% fat)	Skim milk (0.01% fat)	Cream (32% fat)
per cent	*mmg./ml.*	*mmg./ml.*	*mmg./ml.*
0.1	2.6	1.2	5.6
2.5	63.0	31.2	136.6
100	2080	1320	2630

ardized to 32 per cent fat, and on the skim milk. The averages of the data, shown in table 2, indicate that the enzyme is highly concentrated in cream and considerably diluted in the skim milk. It was found also in numerous tests that the enzymic activity varies considerably in different milks, some whole milks yielding values as high as 1500 units of color per 0.5 ml.

The method is suitable for detecting phosphatase activity as low as 0.5 unit of color in the test, by means of a photometer or by comparison with standards in butyl alcohol. Therefore, on the basis of comparisons with values found for 0.1 per cent raw in a boiled product, illustrated in table 2, the sensitivity of the test is such that approximately 0.05 per cent (1 pound in 2,000) of a raw in a boiled product can be detected in the case of 4 per cent milk, 0.02 per cent (1 pound in 5,000) in the case of 32 per cent cream, and 0.1 per cent (1 pound in 1,000) in the case of skim milk. The proportion that can be detected in non-ripened cheese (table 1) is approximately the same as that in whole milk.

SUMMARY

A modification of the phosphatase test for detecting under-pasteurization of milk has been developed for testing Cheddar cheese and this modified method has been applied also to fluid milk.

Research on the conditions and substances that cause interferences in the test has been conducted, and the modifications found effective in minimizing the interferences are described. The resulting test has been found to yield results that are more precise for detecting under-pasturization than those obtained with the tests available heretofore.

In tests on whole milk and on non-ripened cheese, contamination of the pasteurized milk with as little as 0.05 per cent of raw milk (1 pound in 2,000) can be detected. The test applied to cheese has been found reliable for detecting under-pasteurization, regardless of the age of the cheese.

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BACTERICIDAL PROPERTIES OF SOME SURFACE-ACTIVE AGENTS*

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INTRODUCTION

During the last twenty years there has been a gradual increase in the use of chemical sterilizing solutions in dairy plants and on dairy farms. Until recently, materials containing chlorine were the only recognized chemical sterilizing agents for dairy use. Although chlorine has served the dairy industry fairly well in the past, it has some well-known limitations which are of sufficient importance to have stimulated the search for better sterilizing agents. Shortly before World War II the literature called attention to the germicidal properties of some surface-active agents and their possible usefulness in the whole field of sanitation. In general the mode of action of surface-active materials on bacteria is believed to be by a disorganization of the cell membrane, and also by the denaturation of certain proteins essential to metabolism and growth. The present investigation was undertaken primarily for the purpose of evaluating the germicidal potency for dairy use of many surface-active materials and also to evaluate the germicidal stability of these products.

EXPERIMENTAL

The general plan of this study was to secure from manufacturers a reasonably large number of surface-active materials (6) representing various types, and to determine their germicidal properties under conditions simulating dairy practice. The variety of these compounds is so great that the number tested had to be limited. In order to obtain an indication of any relationship that might exist between the approximate chemical structure and the germicidal properties (1, 2, 3, 7) the samples selected were classified into what were believed to be similar chemical groups. The compounds were tested for their germicidal properties and grouped, according to their effectiveness, into three groups: effective, moderately effective, and ineffective. The germicidal properties of the compounds were then correlated with their chemical structure (4). Those materials found to be effective or moderately effective as germicides after two and one-half years storage were used for other studies such as their corrosiveness to metals, while the materials in the ineffective group were discarded.

The investigation was interrupted by the war; hence there was some delay in the first examination of some of the products. However, the inter-

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ruption was advantageous in determining the stability of some of the products.

METHODS

Germicidal properties. The germicidal properties of the surface-active agents were determined by adding 1 ml. of raw milk, containing many bacteria, to 99 ml. of the solution being tested. Immediately after the inoculum was added, each bottle was shaken rapidly 25 times. After the milk had been in contact with the test solution for 5 minutes at room temperature, proper dilutions were made and 1-ml. quantities were plated according to the Standard Method for Milk Analysis (5) procedure. The method used for evaluating the germicidal properties of the surface-active agents was such that the test was carried out in an environment where bacteria can live. The bacteria in the milk used for the inoculum were increased by adding manure, stable bedding material, and soil to the milk. No doubt the flora in the various experiments varied, but they did represent those forms that the germicide must combat in actual dairy practice, which is an important consideration. In this study, where many materials about which so little is known were being tested, it was impractical to add an inactivator to prevent a bacteriostatic action in the plate. The omission of the inactivator is not serious, however, because the chief purpose of the study was to weed out those materials which are germicidally ineffective. Therefore, if a material was ineffective in the absence of an inactivator, it is reasonable to assume that it might have been more ineffective had an inactivator been used. Where the manufacturer recommended the concentration of the surface-active material to be employed, the recommendations were followed; otherwise, the material was used in 0.5 per cent concentration by weight.

Corrosive action on metals. The corrosive properties of the surface-active materials were determined on strips, 2.5 inches by 1 inch, of the following metals: 18–8 stainless steel, monel, tin (4X), and copper tinned on one side only. The metal strips were washed, polished, washed in alcohol and ether, dried, and weighed. Each metal strip was placed in a glass-stoppered bottle (125 ml. capacity) containing 100 ml. of the solution being tested. The test solutions were of the same strength as used for the germicidal tests. The bottles were agitated for three days at room temperature by the use of a revolving agitator driven at 24 r.p.m. At the end of three days the metal strips were removed from the test solutions, rinsed in water, alcohol and ether, dried and reweighed to determine any loss in weight. The appearance of the metal strips and of the solutions, after the test, was also noted.

Hydrogen-ion concentration. The pH value of solutions of the various surface-active agents was determined with the Beckman pH meter, using a glass electrode. On some samples consistent pH determinations were difficult to obtain.

PRESENTATION OF RESULTS

Chemical classification. Forty-two surface-active agents were obtained from 14 different manufacturers. From the manufacturer's descriptive literature, and from other sources in the literature, these surface-active agents were classified in 12 groups as shown in table 1. It may be pointed out that the greater number of the surface-active agents investigated were classed as quaternary ammonium compounds or alkyl aryl sulfonates (4). The number of agents in the other groups are more evenly distributed.

Germicidal properties. The concentration at which each material was used, the inoculum employed, and the percentage survival of bacteria are given in table 2. Because of the well-known inherent limitations of the agar plate method, the percentage survival data should be considered as trends and not as absolute values. While the test for each material was repeated at least three times, the data reported are not averages but are the data of single trials which conformed most closely to the general trends. The 42 agents investigated are also segregated in table 2 into three arbitrary groups according to their effectiveness as sterilizing agents. Group I are the agents which had a bacterial survival of zero to 0.5 per cent inclusive, and this is considered as being very effective. Group II are the agents which had a bacterial survival greater than 0.5 per cent and up to 3 per cent inclusive. This is considered as being only moderately effective. Group III are the agents which had a bacterial survival greater than 3 per cent, and this is considered to be ineffective from a practical viewpoint.

TABLE 1

Classification of the various surface-active agents investigated

	Designation	Total for each classification
Quaternary ammonium compounds	24 25 26 27 28 29 30 31 42	9
Phosphonium compounds	38 39 40	3
Substituted phenols	20 21 41	3
Alkyl aryl sulfonates	1 4 13 15 16 17 18 19 22 23	10
Aryl alkyl polyether sulfonate	33	1
Aliphatic sulfonates	2	1
Aryl alkyl polyether alcohol	35	1
Esters:		
Polyoxyalkylene of fatty acids	9 10	2
Aliphatic sulfates	32	1
Aryl alkyl polyether sulfate	34	1
Monoesters of polyhydroxy compounds	6 7 8 11	4
Unknowns	3 5 12 14 36 37	6
Total		42

From table 2 it is apparent that out of 42 surface-active agents investigated, 9 were effective sterilizing agents, 9 were moderately effective, and

TABLE 2

Germicidal properties of various surface-active agents

Designation	Concentration used*	Inoculum	Percentage survival of bacteria
Group I—Effective			
24	1 : 1000 dilution	60,000,000	0.0
25	1 : 1000 dilution	60,000,000	0.0
26	1 : 1000 dilution	60,000,000	0.0
27	1 : 1000 dilution	60,000,000	0.0
28	1 : 1000 dilution	46,000,000	0.0
38	0.5%	46,000,000	0.0
39	0.5%	46,000,000	0.0
40	0.5%	46,000,000	0.0
42	1 : 500 dilution	113,000,000	0.0
Group II—Moderately effective			
2	0.5%	46,000,000	1.2
16	0.5%	1,287,000,000	0.8
17	0.5%	1,287,000,000	1.5
18	0.5%	1,287,000,000	1.4
19	0.5%	1,287,000,000	1.4
20	0.5%	46,000,000	2.6
21	0.5%	46,000,000	3.0
22	0.5%	46,000,000	2.4
41	0.5%	231,000,000	1.3
Group III—Ineffective			
1	0.5%	231,000,000	7.8
3	0.5%	231,000,000	46.1
4	0.5%	231,000,000	71.2
5	0.5%	231,000,000	50.9
6	1 : 400 dilution	326,000,000	100.0
7	1 : 400 dilution	326,000,000	100.0
8	0.5%	326,000,000	100.0
9	0.5%	326,000,000	62.6
10	0.5%	326,000,000	78.0
11	0.5%	326,000,000	29.6
12	0.5%	231,000,000	34.1
13	0.5%	231,000,000	22.8
14	0.5%	231,000,000	14.9
15	0.5%	231,000,000	8.4
23	0.5%	231,000,000	13.5
29	1 : 1000 dilution	46,000,000	28.3
30	1 : 1000 dilution	326,000,000	55.2
31	1 : 1000 dilution	326,000,000	20.2
32	0.5%	113,000,000	77.9
33	0.5%	231,000,000	83.4
34	0.5%	231,000,000	36.6
35	0.5%	231,000,000	49.9
36	0.5%	46,000,000	37.0
37	0.5%	46,000,000	14.3

* Percentages and dilutions are based on the product as received and not on the basis of the active ingredient.

24 were ineffective. It is known that the germicidal property of a material may increase with an increase in concentration, and in some instances with a decrease in concentration. Therefore, the agents in Group II and III,

which were used at a concentration of 0.5 per cent, were retested at a concentration of 1.0 per cent and 0.05 per cent. In no instance was the group classification changed by the increase or decrease in concentration.

Table 3 shows the correlation between sterilizing property and chemical composition for the 42 agents investigated. It will be noted that the effective group includes only quaternary ammonium compounds and phosphonium compounds. The moderately effective group includes substituted phenols, alkyl aryl sulfonates, and aliphatic sulfonates. One or more of the agents of each class are represented in the ineffective group, with the exception of phosphonium compounds, substituted phenols, and aliphatic sulfonates. It is highly probable that the three ineffective quaternary ammonium compounds were partly inactivated by the manufacturers through the addition of foreign substance to the compounds.

TABLE 3

The effectiveness of the different classes of materials as sterilizing agents

	Number of materials in			
	Group I Effective	Group II Moderately effective	Group III Ineffective	Total
Quaternary ammonium compounds	6	0	3	9
Phosphonium compounds	3	0	0	3
Substituted phenols	0	3	0	3
Alkyl aryl sulfonates	0	5	5	10
Aryl alkyl polyether sulfonate	0	0	1	1
Aliphatic sulfonates	0	1	0	1
Aryl alkyl polyether alcohol	0	0	1	1
Esthers:				
Polyoxyalkylene of fatty acids	0	0	2	2
Aliphatic sulfates	0	0	1	1
Aryl alkyl polyether sulfate	0	0	1	1
Monoesters of polyhydroxy compounds	0	0	4	4
Unknowns	0	0	6	6
Totals	9	9	24	42

Stability. Approximately one and one-half years after the first tests were made, the germicidal property and the hydrogen-ion concentration of the agents in groups I and II were checked. The stability of the agents in group III was not determined because they were eliminated in the first examination as unsuitable chemical sterilizers for dairy use. From the time they were received until the last tests were made, the agents were stored in the containers in which they were received, at room temperature away from direct sunlight. The results obtained are given in table 4. It will be noted that, with one exception, all of the agents found to be effective in the first test were still effective after two and one-half years. No. 26, the exception, had lost enough of its germicidal property during storage for reclassification as only moderately effective. It will also be noted, from table 4, that all of the agents which were found to be moderately effective in the first test had lost enough of their germicidal properties during storage to be reclassified as ineffective. Since stability is one of the major requirements of a sterilizing agent for dairy use, only those agents remaining in the effective and moderately effective groups after two and one-half years storage were considered for further study.

An examination of the hydrogen-ion concentration data in table 4 reveals no correlation between pH, germicidal property, and stability of the agents.

TABLE 4

Stability of the effective and moderately effective sterilizing agents

Designation	Within 1 year after receiving samples		Two and one-half years after receiving samples	
	Percentage survival of bacteria	pH	Percentage survival of bacteria	pH
Group I—Effective sterilizing agents				
24	0.0	6.1	0.0	6.7
25	0.0	4.7	0.0	5.2
26	0.0	6.1	0.7	6.3
27	0.0	5.5	0.0	5.2
28	0.0	5.5	0.0	5.7
38	0.0	6.8	0.0	6.9
39	0.0	7.4	0.0	7.8
40	0.0	6.0	0.0	6.3
42	0.0	6.5	0.0	6.0
Group II—Moderately effective sterilizing agents				
2	1.2	3.7	24.7	3.6
16	0.8	7.3	28.9	6.6
17	1.5	6.8	100.0	7.5
18	1.4	6.8	100.0	6.6
19	1.4	6.8	100.0	6.9
20	2.6	7.2	62.5	7.7
21	3.0	8.1	56.9	8.1
22	2.4	8.0	57.5	9.1
41	1.3	11.1	14.3	11.3

Corrosive effect on metals. Only those agents remaining effective and moderately effective as sterilizing agents after two and one-half years storage were used for the corrosion of metals test. The corrosive effect of 6 quaternary ammonium compounds, 3 phosphonium compounds, and a chlorine product on four metals is shown in table 5. Since chlorine is so widely used it is included as a standard. None of the agents showed sufficient corrosion on 18–8 stainless steel or monel metal to be of any practical significance. As judged by loss in weight of metals, the quaternary ammonium compounds and phosphonium compounds were less corrosive than chlorine to tin (4X) or to copper tinned on one side only. Tin appeared to be more corroded in the presence of copper than when plated on steel. The correlation between corrosion, as judged by appearance and by loss in weight, is not very close.

TABLE 5

Corrosion of metals by various chemical sterilizers

Sterilizing agent	Amount used*	Loss (−) or gain (+) in weight per square foot area of metal				Extent of tarnishing or corrosion†			
		18–8 stainless steel	Monel	Tin (4X)	Tinned copper	18–8 stainless steel	Monel	Tin (4X)	Tinned copper‡
Quaternary ammonium compounds									
24	1 : 1000 dilution	−0.0058	−0.0346	−0.0461	−0.1901	−	−	++	C + T ++++
25	Same	−0.0115	−0.0518	−0.1037	−0.2477	−	−	++	C + T ++++
26	Same	−0.0115	−0.0461	−0.0288	−0.1843	−	−	++	C ++ T +++
27	Same	0.0000	−0.0691	−0.0864	−0.0403	−	−	++	C + T ++
28	Same	−0.0058	−0.0058	−0.4205	−0.2534	−	−	++	C ++ T +++
42	1 : 500 dilution	−0.0115	−0.0115	−0.1210	−0.2074	−	−	++	C ++ T +++
Phosphonium compounds									
38	0.5%	+0.0576	+0.0230	0.0000	−0.3728	−	−	+	C ++ T ++
39	Same	+0.0461	+0.0058	+0.0115	−0.0576	−	−	+	C + T ++
40	Same	+0.0230	−0.0058	−0.0461	−0.0230	−	−	+	C + T ++
Calcium hypochlorite	200 p.p.m. of Cl	−0.0115	−0.1037	−0.4378	−0.8410	−	−	++	C + T +++++

* Percentages and dilutions are based on the product as received and not on the basis of the active ingredient.

† Degree of tarnishing or corrosion ranges from (−), representing no change, or (+) indicating very slight dulling of luster to (+++++) indicating very badly tarnished or corroded.

‡ Copper strips tinned on one side only.

The phosphonium compounds in many instances deposited a light waxy film on the surface of the metals. This film was not easily removed with alcohol or ether, which no doubt accounts for the slight increase in weight for some of the metals. While it is difficult to give a practical interpretation of the losses in weight for tin plate and tinned copper, it appears that when the surface-active agents are compared with chlorine they would be satisfactory for dairy use from the viewpoint of corrosion.

Solubility, odor, taste, and color. Observations under this heading are reported only for those surface-active agents which were effective germicides after two and one-half years storage. All of the quaternary ammonium compounds investigated are readily soluble, have no objectionable odor or taste, and are practically colorless. However, all three of the phosphonium compounds went into solution with such difficulty that this property would probably exclude them from use as a dairy sterilizing agent. They are not objectionable from the standpoint of odor and taste. The color of their solutions is very cloudy or milky, which is not as desirable as a colorless sterilizing solution.

DISCUSSION

In this study the major considerations were germicidal properties, corrosiveness to metals, stability, solubility, odor, taste, and color. From these tests it appears that the quaternary ammonium compounds are the only group which show promise of making satisfactory sterilizing agents for dairy use. Further studies of the quaternary ammonium compounds will show how these new sterilizing agents can be used to the best advantage to the dairy industry.

The manufacturers of many of the products employed in this investigation do not make any claim that the product is a suitable dairy sterilizing agent. Therefore, it cannot be overemphasized here that the germicidal classification given as a result of this study is in no way a rating of the products for the uses they are now sold for. Since a dairy cleaner should also have considerable germicidal properties, possibly some of the surface-active agents which are not really effective sterilizing agents may have sufficient germicidal properties to be useful as ingredients for dairy cleansing purposes.

SUMMARY

1. The germicidal properties of the following 42 surface-active agents have been investigated: 9 Quaternary ammonium compounds, 3 Phosphonium compounds, 3 Substituted phenols, 10 Alkyl aryl sulfonates, 1 Aryl alkyl polyether sulfonate, 1 Aliphatic sulfonate, 1 Aryl alkyl polyether alcohol, 2 Polyoxyalkylene of fatty acids, 1 Aliphatic sulfate, 1 Aryl alkyl polyether sulfate, 4 Monoesters of polyhydroxy compounds, and 6 Unknowns.

2. In the first examination 9 were effective sterilizing agents, 9 were moderately effective, and 24 were ineffective.

3. The effective group included only quaternary ammonium and phosphonium compounds.

4. The moderately effective group included substituted phenols, alkyl aryl sulfonates, and aliphatic sulfonates.

5. One or more of the materials of each class are represented in the ineffective group, with the exception of phosphonium compounds, substituted phenols, and aliphatic sulfonates.

6. All of the 9 surface-active agents, with one exception, found to be effective by the first test, remained effective after two and one-half years storage.

7. All of the 9 surface-active agents, found to be moderately effective by the first test, had lost much of their germicidal properties after two and one-half years storage.

8. There was no correlation between pH value, germicidal property, and stability of the products.

9. The 6 quaternary ammonium compounds which remained effective or moderately effective as sterilizing agents after two and one-half years storage were satisfactory in so far as corrosiveness to metals, solubility, odor, taste, and color are concerned.

10. All three phosphonium compounds were satisfactory in so far as corrosiveness to metals, odor, and taste are concerned, but they are objectionable because they do not go into solution easily and also form cloudy solutions.

CONCLUSIONS

Of the 42 surface-active agents investigated, only the quaternary ammonium and phosphonium compounds had sufficient germicidal properties and stability for a good sterilizing agent for dairy use. The quaternary ammonium compounds go into solution readily, are practically non-corrosive to metals, odorless, tasteless, and colorless, all of which are desirable properties. The phosphonium compounds also are non-corrosive to metals and have no serious objectionable odor or taste, but they do not go into solution readily and produce cloudy solutions, which are undesirable properties.

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THE EFFECT OF VITAMIN SUPPLEMENTS UPON SURVIVAL OF NEW-BORN CALVES*

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INTRODUCTION

Liebscher (1) reported in 1930 that calves which had previously been given colostrum, could be raised on skim milk and cod-liver oil, but they failed to gain in weight as rapidly as calves fed whole milk. Numerous reports have appeared regarding the importance of colostrum to the newborn (2, 3, 4). Phillips *et al.* (5) fed calves which had never received colostrum on a ration of skim milk and found that some of the animals would survive. It was also found that ascorbic acid and nicotinic acid would increase the survival rate of calves on their skim milk vitamin A ration.

The bovine placental membrane appears to be relatively impervious to the passage of vitamin A, as the calf is born deficient in this factor (6, 7, 8). Colostrum generally meets this deficiency for it may contain from 10–100 times the amount of vitamin A that is present in normal milk (6, 9); however, Stewart and McCallum (10) have observed a very wide variation in colostral vitamin A among cows of the same breed receiving similar rations. Under our present system of dairy calf management it is probable that the young calf may under certain conditions receive inadequate amounts of this vitamin.

This study was made to determine the amount of vitamin A needed by the calf fed from birth on a skim milk ration, and to determine the benefit to the calf of additional factors that are also known to occur in variable amounts in colostral milk.

EXPERIMENTAL

Holstein calves weighing from 90–110 lbs. were obtained from local farms before having access to colostrum. These calves were selected and alotted in such a manner that they alternately fell into their respective groups in order to eliminate as much as possible the factor of seasonal influence and variables attendant thereto. Skim milk was fed twice daily at the rate of twelve pounds of milk per hundred pounds of body weight per day. Since it has been shown (5) that under certain conditions the administration of

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[1] Now at the Texas Agricultural and Mechanical College.

ascorbic acid is beneficial, 250 mg. of this vitamin were given orally to all calves once each day. Standardized vitamin A concentrates were given in gelatin capsules at levels of 10,000 and 25,000 I.U. per calf per day as indicated.

In preliminary experiments with the skim milk ration it was found that most calves were lost during the first week of life. Little difficulty was encountered with older calves, hence fourteen days was taken as an adequate length of time to measure the effectiveness of a ration in meeting the requirements of the very young calf. For this reason the survival figures are given on the basis of a fourteen-day experimental period.

Calves fed the basal ration normally exhibited a looseness of the bowels regardless of the dietary supplements employed. If the looseness reached a severe stage, accompanied by a rise in body temperature (104–105° F.), 2–4 grams of sulfathiazole were given for one day and lighter doses thereafter as the condition of the calf indicated.

Blood was taken from the jugular vein at birth and before feeding, at 1½, 7, and 14 days. The samples were cooled immediately and plasma vitamin A determinations were made according to the method of Kimble (11).

I. *The effect of vitamin A level fed.* In agreement with previous observations (5) the blood plasma vitamin A level of the new born calf was low. Following the ingestion of colostrum there was an immediate rise from 4 micrograms per 100 ml. to a level of 10 micrograms or more per 100 ml. of blood. It is seen from the data in table 1 that the new-born calves did not develop normal blood plasma vitamin A levels within a thirty-six-hour period when only 10,000 I.U. of vitamin A were fed with the basal ration, while the administration of 25,000 I.U. of vitamin A per day produced blood plasma vitamin A levels comparable to the calves fed colostrum.

TABLE 1

The effect of vitamin supplements upon the occurrence of severe diarrhea and survival of young calves

Lot	I	II	III	IV	V	VI
	10,000 I.U. of vit. A		25,000 I.U. of vit. A			
Treatment	Vit. A only	+ Nicotinic acid	Vit. A only	+ Nicotinic acid	+ Nicotinic acid + biotin	Colostrum and whole milk
No. calves	3	6	13	16	7	4
Blood plasma vitamin A concentration µg./100 ml.						
Birth	2	5	4	4		4
1–2 days	4.5	6	9	10	..	10
6–14 days		12	11	11		13
No. cases diarrhea	3	5	9	11	5	
No. given sulfathiazole	3	5	7	9	4	..
No. calves survived	1	3	9	12	6	4
Per cent survival	33	50	69	75	86	100

The data in table 1 further indicate that the level of vitamin A fed influenced the rate of incidence of severe diarrhea as eight out of nine calves (89 per cent) receiving 10,000 I.U. of vitamin A per day showed severe diarrhea, while only 25 out of the 36 calves (69 per cent) receiving 25,000 I.U. of vitamin A showed this symptom. The level of vitamin A administration seemed to have a direct bearing upon the capacity to survive. Only 4 out of the nine calves fed 10,000 I.U. of vitamin A per day survived the experimental period whereas 27 out of 36 (75 per cent) of the calves fed 25,000 I.U. of vitamin A survived.

II. *The effect of nicotinic acid and biotin.* The data of table 1 show that neither nicotinic acid nor its amide when given orally (50 mg. daily) increased the survival rate of calves fed the skim milk ration fortified with adequate vitamin A. There may have been a slight beneficial effect upon the survival rate when only 10,000 I.U. of vitamin A were fed but the number of calves used was too few to permit a definite conclusion on this point.

Since the biotin content of colostrum milk was found to be low and somewhat variable (12) the calves in lot V (table 1) were given biotin to determine its effect upon survival and general health of the calves. 150 micrograms of biotin were given subcutaneously as well as orally each day in addition to the basal ration fortified with vitamin A and niacin. There was no marked effect from the administration of biotin either in the prevention of diarrhea or on the survival rate of the calves.

The effect of season. The data in table 2 clearly indicate a marked seasonal variation in the occurrence of severe diarrheas and survival of young calves fed the skim milk basal ration. During the period of January 1, to June 1, 86 per cent of the calves showed symptoms of severe diarrhea. In contrast only 43 per cent of the calves developed severe diarrheas throughout the period from June 1 to January 1. This type of diarrhea usually occurred during the first week of life.

TABLE 2

The effect of season on the occurrence of diarrhea and survival of young calves

Season		25,000 I.U. of vitamin A		
		Vit. A only	Vit. A + nicotinic acid	Vit. A + nicotinic acid + biotin
Jan. 1 to June 1	No. calves	10	10	2
	No. cases diarrhea	9	8	2
	No. calves survived	6	7	1
June 1 to Dec. 31	No. calves	3	6	5
	No. cases diarrhea	0	3	3
	No. calves survived	3	5	5

The data in table 2 also demonstrate an effect of season on the survival rate of calves fed the skim milk basal with vitamin supplements as indicated. In the period from June 1st through December, 13 out of 14 calves (92 per cent) survived the experimental period, while of the calves in the January–June group that received identical treatment 14 out of 22 (64 per cent) survived.

DISCUSSION

The vitamin A requirement of the new-born calf appears to us to be considerably higher (5 to 10 ×) during the first few days of life than that previously established for heifers 3–6 months of age. These data support such an interpretation but they also raise the question of the efficacy of the source of vitamin A. Specifically; is one unit of vitamin A in fish-liver oil concentrate equivalent to one unit of vitamin A in colostrum milk? In these experiments it has been assumed that they were but we grant that the possibility of conjugated forms of vitamin A present in colostrum may be superior to the concentrated fish-liver oil vitamin A used. The data do indicate, however, that the vitamin A requirement of the new born calf fed a highly concentrated vitamin A fish-liver oil lies between 10,000 and 25,000 I.U. per 100-lb. calf per day. These results are in general agreement with those suggested by Lewis and Wilson in 1945 (13).

These data throw considerable light upon the specific effect of niacin in relation to blood plasma vitamin A. In 1943 Phillips *et al.* (5) presented data which indicated that B complex mixtures containing niacin favorably influenced the recovery of ingested vitamin A in the blood plasma of the calf. Subsequent observation on the administration of 5,000 I.U. of vitamin A per calf per day has usually shown this level of vitamin A ingestion to be more effective when niacin was also given. In the present study the favorable effect of niacin disappeared when higher levels of vitamin A were ingested. The niacin effect was only slightly in evidence, if at all, when 10,000 I.U. of vitamin A were given. These data point to the conclusion that in the presence of adequate vitamin A (25,000 I.U. per calf per day) supplementary amounts of niacin are unnecessary.

A seasonal effect is quite apparent from the data presented in table 2. With the fortified skim milk basal ration summer ''dropped'' calves showed a lower incidence of severe diarrhea and a higher rate of survival than winter calves. It does not seem likely that the trace of fat present in the skim milk could account for this difference.

Many other factors are recognized which may be involved in the summer–winter differential in survival. Factors such as crowding, ventilation, temperature, drafts, etc., are far from being alike for the two periods. Hence the difference noted in survival cannot be wholly attributed to nutrition at this time. In view of the demonstrated superiority of summer vs. winter milk for rats (14), however, it seems not unlikely that either dietary factors present in the skim milk, or the intrauterine nutrition or fetal storage of metabolites was favorably affected by season. Storage of additional vitamin A in the fetal liver would be highly important in this regard. A few attempts to obtain blood plasma vitamin A levels of 10 + µg./100 ml. in the new-born calf by feeding moderate amounts of high vitamin A shark-liver oil to their dams did not meet with success. It is postulated as a working hypothesis, that some factor, or factors present either in "summer skim milk" or in intrauterine life or both favorably influence this seasonal survival.

SUMMARY

A study of the effect of vitamin supplements upon survival of new-born calves fed a skim milk basal ration from birth has been made. It appears that under these conditions new-born calves require vitamin A in excess of 10,000 I.U. per day when severe diarrhea and ability to survive were used as the criteria for establishing approximate requirements. Twenty-five thousand I.U. of vitamin A per day in the form of fish-liver oil concentrate greatly enhanced the chance of survival of calves fed the skim milk ration. In the presence of adequate vitamin A ingestion the favorable supplementary effect of niacin was found to be negligible. The blood-plasma vitamin A concentration followed previous observations, *i.e.*, on the average the new-born calf has a very low blood plasma vitamin A level and that normal concentrations in the neighborhood of 9–10 µg./100 ml. or more must be quickly attained (1–2 days) for a favorable chance of survival.

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STUDIES ON MILK FEVER IN DAIRY COWS.[1] II. THE EFFECT OF VITAMIN D ON SOME OF THE BLOOD CHANGES IN NORMAL AND MILK FEVER COWS AT PARTURITION

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Many of the usual blood changes occurring at parturition and in milk fever have been extensively investigated. Since Little and Wright (24) reported low blood calcium values in cows with milk fever much attention has been given to various mineral constituents of the blood. It appears that when an explanation is found for the changes in the mineral constituents of the blood and a way to prevent those changes can be devised, the mystery of the milk fever syndrome will be solved.

Since the report of Little and Wright (24) numerous reports have been submitted dealing with total calcium (1, 2, 10, 11, 12, 14, 15, 17, 24, 25, 27, 32, 33, 36) and diffusible calcium (15, 27, 30, 33) in the blood of normal and milk fever cows at parturition. All are in agreement that a marked decrease in both total and diffusible calcium occurs in milk fever. A sharp drop in blood inorganic phosphorus in milk fever has been repeatedly reported (2, 11, 14, 15, 27, 29, 30, 33, 36).

In contrast to serum calcium and phosphorus, serum magnesium is said to increase during milk fever (1, 2, 15, 23, 29, 30, 31, 33).

The work of Mattick and Little (26) suggests some benefit in preventing the fall of blood calcium and phosphorus at parturition as the result of feeding cod-liver oil.

The present paper is a report of studies covering the effect of irradiated yeast feeding on some of the blood changes at parturition and in milk fever, made in connection with the experiment described in the preceding paper (20) involving the effect of feeding vitamin D in the form of irradiated dry yeast on the incidence of milk fever.

EXPERIMENTAL

Method of Analysis

Calcium: Serum calcium was determined by the method of Clark and Collip (9).

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[2] N. E. Van Demark assisted in obtaining the data from the Ohio State University herd.

Phosphorus: Serum phosphorus was determined by the method of Briggs (7).

Magnesium: Serum magnesium was determined by the Briggs (6) method.

Phosphatase: Serum phosphatase was determined by the Bodansky (3) method. The results are expressed as units per 100 ml. of serum, a unit being defined as "equivalent to 1 mg. of phosphorus liberated from a sodium glycerophosphate substrate as the phosphate ion during the first hour at a pH of 8.6 and at 37° C."

Serum protein: Total serum protein was measured by the Falling Drop Method of Kagan (21) using a Kagan Proteinometer.

Vitamin D: Samples of whole blood were dried over night at 100° C. and finely ground. The appropriate amount was weighed out and mixed with 35 mg. of rachitic ration (Steenbock and Black's No. 2965) and fed to rachitic rats. After this amount of feed was consumed the rats were continued on the rachitic ration until the tenth day. The rats were then sacrificed and the ulna and radius removed. The distal end of the ulna was then split and after staining with silver nitrate the bones were photographed and the healing response was measured by the relative amount of calcification at the metaphysis. The per cent healing was determined by dividing the actual healing by 4, the value which represented complete healing. Thus an average healing response of 1 would be 25 per cent, etc.

Vitamin A and carotene: Plasma vitamin A and carotene were determined by the method of Kimble (22). In samples containing carotene in excess of 300 micrograms per 100 ml. the vitamin A was determined by the method of Boyer, Phillips, and Smith (4). The carotene and vitamin A in colostrum were determined by using essentially the same method described by Boyer, Spitzer, Jensen, and Phillips (5) except that the saponification was carried out by refluxing in a boiling water bath for ten minutes instead of the cold saponification. A few minor modifications were also made in the extraction process.

Ascorbic acid: Plasma ascorbic acid was determined by the macro-method of Mindlin and Butler (28).

An Evelyn Photo-electric Colorimeter was used for all colorimetric determinations.

Experiment Involving Type 7F Irradiated Dry Yeast—1941

In a preliminary trial, 1 million units of vitamin D were fed daily to cows in the form of type 7F irradiated dry yeast[3] for four weeks before and one week following parturition. Calcium and phosphorus determinations were made on the blood serum before yeast feeding started, within 12 hours before and within 12 hours after parturition. Samples of whole blood were collected

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[3] All yeast was supplied by Standard Brands Incorporated.

TABLE 1

The effect of feeding one million units of vitamin D daily on the serum calcium and phosphorus content of cows' blood

Cow No.	4 weeks pre-fresh		12 hr. pre-fresh		12 hr. post-fresh		Milk fever	
	Ca mg. %	P mg. %	Ca mg. %	P mg. %	Ca mg. %	P mg. %	Ca mg. %	P mg. %
				Control cows				
4	10.95	5.18	9.41	4.67	8.20	6.02		
5			11.13	5.81	10.52	4.35		
13*	10.93	4.65	11.23	6.06	8.92	2.60	5.46	1.51
14	10.83	4.14	10.93	5.18	8.70	3.83		
18*	12.14	5.43	11.74	4.98	7.49	2.86	5.87	2.46
				Yeast-fed cows				
2	10.73	5.71	11.33	5.56	11.74	5.75		
7	11.74	5.65	10.63	5.15	12.14	5.78		
10*	10.93	5.71	10.32	5.00	9.72	3.65		
11	10.93	5.56	10.83	4.39	8.91	5.26		
15	10.93	4.55	11.84	7.41	6.88	4.63		
17*	11.13	5.72	11.92	7.62	8.30	4.46		

* These cows had previous histories of milk fever.

and dried for vitamin D assay at the same times. A similar group of cows receiving no irradiated yeast supplement served as controls. The results of this experiment are presented in tables 1 and 2.

No marked change due to the irradiated yeast feeding occurred in the serum-calcium and serum-phosphorus levels of the blood although both serum calcium and serum phosphorus of the yeast-fed cows averaged slightly higher after freshening (table 1).

Table 2 shows that only one-fourth as much dried blood from the yeast-fed cows as from the control cows was required to produce the same healing response, indicating that the blood of the yeast-fed cows contained approximately 4 times the vitamin D potency of the controls.

TABLE 2

Relative vitamin D potency of dried whole blood from normal and milk fever cows

Group	No. of cows	Milli-grams of dried blood	Per cent healing, 4 weeks pre-fresh	Per cent healing, 12 hours pre-fresh	Per cent healing, 12 hours post-fresh	Per cent healing, milk fever
			Normal			
Control	3	1,000	10.0	5.0	5.0	
Yeast	6	250	5.0*	9.7	12.8	
			Milk fever			
Control	2	1,000	10.0	5.0	9.0	6.3

* 1,000 mg. of dried blood.

TABLE 3

The effect of feeding one million units of vitamin D daily on the blood serum calcium, phosphorus, magnesium and phosphatase of normal and milk fever cows

Group	No. of cows	Blood constituent (mg. per cent)	4 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Jerseys, normal parturitions							
Control	25	Calcium	11.10	10.15	9.40	...	10.40
Yeast	30	Calcium	10.70	10.42	9.40		10.53
Control	25	Phosphorus	5.70	4.08	3.59		4.65
Yeast	30	Phosphorus	5.59	4.83	3.75		5.37
Control	4	Magnesium	2.14	2.40	2.30		2.13
Yeast	2	Magnesium	2.90	2.62	3.03		1.78
Control	3	Phosphatase	5.07	5.03	2.55		3.17
Yeast	7	Phosphatase	5.33	2.97	3.99		2.58
Jerseys, milk fever parturitions							
Control	13	Calcium	10.72	10.05	7.98	4.95	10.30
Yeast	12	Calcium	10.80	9.50	7.38	4.76	10.30
Control	13	Phosphorus	5.89	4.68	2.86	1.47	4.83
Yeast	12	Phosphorus	5.38	4.90	3.32	1.73	5.33
Control	2	Magnesium	1.79	2.51	3.17	3.57	1.87
Yeast	5	Magnesium	2.11	2.82	3.08	3.57	2.18
Control	3	Phosphatase	5.35	2.69	2.00	0.83	2.02
Yeast	0	Phosphatase					
Holsteins, normal parturitions							
Control	23	Calcium	10.39	9.81	10.40		11.30
Yeast	12	Calcium	11.40	11.30	10.69		11.66
Control	23	Phosphorus	5.65	4.79	4.70		5.43
Yeast	12	Phosphorus	5.30	5.57	4.38		5.50
Control	5	Phosphatase	3.03	3.39	3.98		4.03
Yeast	1	Phosphatase	1.70	2.02	2.25		2.40
Holsteins, milk fever parturitions							
Control	0	Calcium					
Yeast	1	Calcium	10.40		9.00	5.80	9.80
Control	0	Phosphorus					
Yeast	1	Phosphorus	5.48		5.95	5.58	6.73
Ayrshires, normal parturitions							
Control	10	Calcium	10.28	9.59	8.21		10.10
Yeast	8	Calcium	10.24	9.44	8.79		10.90
Control	10	Phosphorus	5.15	4.49	4.22		4.56
Yeast	8	Phosphorus	5.68	5.78	5.18		6.06
Control	2	Phosphatase	4.61	4.10	4.40		3.99
Yeast	1	Phosphatase	5.70	5.03	6.07		2.30
Ayrshires, milk fever parturitions							
Control	1	Calcium	10.58		8.26	..	9.27
Yeast	2	Calcium	10.23	10.60	6.20	5.42	10.43
Control	1	Phosphorus	3.41		2.97	...	3.08
Yeast	2	Phosphorus	5.73	4.61	3.62	2.34	4.47
Control	0	Phosphatase	. . . .				
Yeast	1	Phosphatase	4.65	4.80		6.39	3.16

TABLE 3 (*Continued*)

Group	No. of cows	Blood constituent (mg. per cent)	4 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Guernseys, normal parturitions							
Control	7	Calcium	11.05	9.79	8.71		10.59
Yeast	6	Calcium	10.48	9.86	8.66		10.17
Control	7	Phosphorus	4.79	4.51	4.55		5.23
Yeast	6	Phosphorus	5.34	4.65	4.16		4.81
Control	0	Phosphatase					
Yeast	1	Phosphatase	4.92	4.15	3.00		4.37
Guernseys, milk fever parturitions							
Control	0	Calcium					
Yeast	2	Calcium	10.73	9.20	7.65	4.84	10.30
Control	0	Phosphorus					
Yeast	2	Phosphorus	5.13	4.30	2.72	1.21	4.94

Experiment Involving Type 9F Irradiated Dry Yeast

Based on these preliminary findings a more extensive experiment was set up as described previously (20). In both the control and yeast-fed groups blood samples were drawn for analyses at the following times: Four weeks before the expected date of parturition, within twelve hours both before and after parturition, and one week after parturition. If milk fever occurred, samples were taken during the attack before treatment, and at frequent intervals during the recovery period.

The results shown in tables 3, 4, and 5 include data obtained from September 15, 1941, to September 15, 1944. Table 3 is a comparison of the serum calcium, phosphorus, magnesium, and phosphatase of yeast-fed and control cows, that freshened normally and with milk fever. Table 4 gives a comparison of the vitamin D potency of the blood of yeast-fed and control cows that freshened normally and with milk fever, as measured by the relative per cent healing elicited by one gram of dried whole blood when fed to rachitic rats.

In order to study possible changes in some of the other blood constituents, determinations were made at the times previously indicated for serum protein and plasma ascorbic acid, vitamin A and carotene. These four constituents were determined in the blood of Jersey cows that freshened normally and with milk fever. The results are shown in table 5. The values found for carotene and vitamin A at parturition are in agreement with those reported by Sutton, Kaeser, and Soldner (35).

High Mineral Ration Plus Various Amounts of Vitamin D

From September 15, 1944, to September 15, 1945, the work was confined to Jersey cows in the Main and Pasture Farm Experiment Station herds and

TABLE 4

The effect of feeding one million units of vitamin D daily on the relative vitamin D content of one gram of whole dried blood. (Per cent healing of rachitic bones)

Group	No. of cows	Per cent healing				
		4 weeks pre-fresh	12 hours pre-fresh	12 hours post-fresh	Milk fever	1 week post-fresh
Jerseys, normal parturitions						
Control	12	26.2	23.8	37.5		31.0
Yeast	15	30.2	56.8	42.5		49.0
Jerseys, milk fever parturitions						
Control	12	19.2	11.8	22.5	18.2	20.5
Yeast	5	26.0	50.0	53.2	44.5	42.5
Holsteins, normal parturitions						
Control	5	42.5	33.2	30.0		44.8
Yeast	4	53.2	56.2	60.0		62.2
Holsteins, milk fever parturitions						
Control	0					
Yeast	1	53.2		43.8	56.2	56.2
Ayrshires, normal parturitions						
Control	7	10.1	32.2	28.5		29.8
Yeast	2	25.0	53.2	56.2		53.2
Ayrshires, milk fever parturitions						
Control	0					
Yeast	1	50.0	62.5		50.0	50.0
Guernseys, normal parturitions						
Control	3	40.8	47.0	40.8		37.5
Yeast	1	22.0	37.5	47.0		34.5
Guernseys, milk fever parturitions						
Control	1	22.0	37.5	47.0		34.5
Yeast	1	50.0	58.2	66.8	50.0	

TABLE 5

Changes in the serum protein and plasma ascorbic acid, vitamin A and carotene content of Jersey cows' blood in normal and milk fever parturitions

Group	No. of cows	Blood constituent	4 wks. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
Normal	7	Serum protein, mg.%	6.63	6.68	6.38	...	6.69
Milk fever	8		6.90	6.18	6.23	6.04	6.48
Normal ...	7	Ascorbic acid, mg. %	0.48	0.49	0.40	...	0.46
Milk fever	8		0.50	0.41	0.47	0.46	0.51
Normal	7	Carotene, microgm. %	757	454	459	...	446
Milk fever	8		726	553	551	525	478
Normal	7	Vitamin A, microgm. %	18.8	16.6	12.9	...	17.2
Milk fever	8		16.4	12.3	11.5	8.6	14.4

TABLE 6

High calcium and phosphorus ration No. 43

	Lb.
Ground corn	140
Ground oats	200
Wheat bran	400
Linseed oil meal	100
Molasses	100
Steamed bone meal	50
Iodized salt	10
Total	1,000

only those cows with at least two previous calvings were included. In order to make sure that calcium and phosphorus were not limiting factors, the ration shown in table 6 was fed during the dry period beginning eight weeks before the due date and continuing for one week after parturition.

Since previous results (tables 1 and 2) had shown no significant differ-

TABLE 7

The effect of various amounts of vitamin D on some of the blood changes in Jersey normal and milk fever cows at parturition

Group*	No. of cows	Blood constituent, mg. per cent	8 wk. pre-fresh	4 wk. pre-fresh	2 wk. pre-fresh	12 hr. pre-fresh	12 hr. post-fresh	Milk fever	1 wk. post-fresh
		Normal parturitions							
I	4	Calcium	10.73	10.29	10.78	10.50	9.10		10.55
II	3	Calcium	11.05	10.80	10.42		9.31		10.83
III	5	Calcium	10.73	10.33	10.85	11.45	9.26		10.46
I	4	Phosphorus	6.13	6.95	6.00	6.50	5.25		6.25
II	3	Phosphorus	6.45	7.49	7.89		6.90		5.05
III	5	Phosphorus	6.58	6.66	8.23	8.64	8.60		6.42
I	4	Magnesium	1.89	1.93	2.09	2.12	2.43		1.75
II	3	Magnesium	2.13	1.90	1.95		2.28		2.04
III	5	Magnesium	2.44	1.74	1.73	1.62	1.86		1.34
		Milk fever parturitions							
I	1	Calcium	9.98	9.24	10.62	7.10	6.40	3.50	8.50
II	3	Calcium	10.07	10.89	10.46		6.86	5.49	10.22
III	1†	Calcium	11.47	11.56	11.52		10.67		Died
I	1	Phosphorus	5.02	8.80	7.36	3.82	2.92	1.63	4.75
II	3	Phosphorus	4.88	6.71	6.86		3.99	3.49	6.61
III	1†	Phosphorus	7.64	7.35	8.40		6.88		Died
I	1	Magnesium	1.81	1.09	1.74	2.56	2.74	2.96	1.25
II	3	Magnesium	2.02	2.26	1.76		2.18	2.55	1.82
III	1†	Magnesium	2.26	2.30	1.25		1.83		Died

* The following amounts of vitamin D were fed daily for four weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

† It is questionable whether this cow had milk fever as the blood picture is not typical and she did not respond to two calcium gluconate injections. She died later of complications.

ence in the calcium and phosphorus content of the blood between yeast-fed and control cows, it was decided to feed two million units of vitamin D daily to a few cows and observe any blood changes which might occur.

The eighteen available cows were divided into three equal groups. All the cows were fed the high mineral ration as previously described. Group I received no supplemental irradiated yeast. Group II received one million units of vitamin D daily in the form of type 9F irradiated dry yeast and Group III was given a similar amount of type 22F irradiated dry yeast which provided two million units of vitamin D daily. The yeast feeding was begun four weeks before the expected date of parturition and continued for one week after parturition. Blood samples were drawn for calcium, phosphorus, magnesium and vitamin D analyses at the same times as in previous experiments except that an additional eight-week pre-freshening and a 2-week pre-freshening sample were included.

One case of milk fever occurred in Group I and one abortion. The blood data of the abortion case are omitted from the averages. Three cases of milk fever occurred in Group II and one questionable case in Group III. The results of the blood analyses are shown in tables 7 and 8.

As a possible means of explaining the blood changes associated with milk fever, as often as possible during this experiment a sample of colostrum was secured before the calf nursed. The colostrum samples were analyzed for

TABLE 8

The effect of various levels of vitamin D intake on the relative vitamin D content of dried whole blood

Group*	No. of cows	Mg. dried blood fed	Per cent healing, 4 wks. pre-fresh	Mg. dried blood fed	Per cent healing, 2 wks. pre-fresh	Mg. dried blood fed	Per cent healing, 12 hr. post-fresh	Mg. dried blood fed	Per cent healing, milk fever	Mg. dried blood fed	Per cent healing, 1 wk. post-fresh
Normal parturitions											
I	2	1,000	46	1,000	56	1,000	48			1,000	48
II	1	1,000	25	500	50	500	63			500	58
III	4	1,000	50	250	61	250	61			250	61
Milk fever parturitions											
I	2	1,000	50	1,000	40	1,000	48	1,000	27	1,000	33
II	3	1,000	44	500	56	500	65	500	63	500	58
III	1	1,000	54	250	61	250	54	250		250	...

* The following amounts of vitamin D were fed daily for 4 weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

TABLE 9

The composition of colostrum from normal and milk fever Jersey cows fed various amounts of vitamin D

Group*	No. of cows	Units of vitamin D daily	Specific gravity	Total solids	Ash, per cent	Fat, per cent	Total protein	Carotene, γ %	Vitamin A, γ %
Normal parturitions									
I	2	None	1.067	19.0	1.27	1.78	13.5	147	68
II	4	1,000,000	1.068	22.0	1.09	2.50	14.8	208	144
III	3	2,000,000	1.061	20.3	1.03	3.20†	13.2	129	138
Average			1.065	20.8	1.11	2.40	14.0	168	126
Milk fever parturitions									
I	1	None	1.070	26.0	1.15	5.85	17.2	285	188
II	2	1,000,000	1.057	17.9	1.03	3.35	11.6†	187	146
III	0	2,000,000							
Average			1.061	20.6	1.07	4.15	14.4	220	160

* The following amounts of vitamin D were fed daily for four weeks before and one week following parturition: Group I—None. Group II—1 million units. Group III—2 million units.

† Only one cow.

the following: total solids, specific gravity, ash, total protein, fat, carotene, and vitamin A. The results are presented in table 9.

DISCUSSION

As is indicated in tables 1, 3, and 7, the feeding of 1 million units of vitamin D did not have any significant effect on the serum calcium and phosphorus levels of the blood at parturition regardless of whether or not milk fever developed. This finding probably explains why no lowering of milk fever incidence was observed at this level of vitamin D feeding, as reported in the first paper of this series (20).

When 2 million units of vitamin D daily were fed (table 6) an increase in both serum calcium and serum phosphorus occurred before parturition. It is possible, therefore, that at still higher levels of vitamin D feeding sufficient increases in blood calcium and phosphorus might be obtained to prevent the decrease of these blood constituents to the milk-fever level. Experiments are now in progress to investigate this possibility.

Of interest is the increase in serum magnesium concurrent with the fall in serum calcium and serum phosphorus in both normal and milk fever freshenings. The rise in serum magnesium is probably a compensatory phenomenon elicited by the fall in calcium and phosphorus and apparently has a marked effect on the symptoms seen during the milk fever attack. At the low level of serum calcium present in milk fever, tetanic symptoms would normally be expected rather than the flaccid, comatose condition usually observed. This is seemingly due to the high serum magnesium in relation to the low serum calcium. On the other hand when nervous symptoms accompany milk fever the serum magnesium is nearly always normal or below normal, as reported by Barker (2).

In this connection some of our experiments, in which $MgSO_4$ was injected intravenously, are of interest. Upon injection of a solution of this salt into normal cows general anesthesia resulted when the serum magnesium reached a level of approximately 7.5 mg. per 100 ml. When the serum calcium was then raised to about 17.5 mg. per 100 ml. by injecting calcium gluconate the cows regained consciousness even though the serum magnesium remained at the same high level. The serum calcium and magnesium were both found to be back to normal levels within 5 hours after injection.

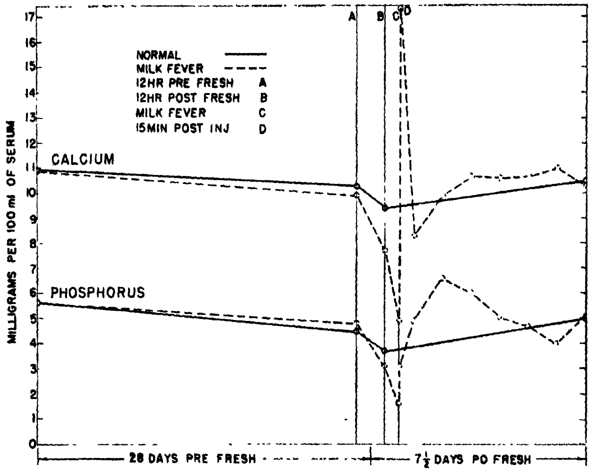

FIG. 1. Changes in the serum calcium and phosphorus of the blood before and after parturition of 55 normal Jersey cows and 25 Jersey cows that developed milk fever. (Both control and yeast fed cows are included.)

It seems, therefore, that the relationship between serum magnesium and serum calcium may be of great importance in the symptomatology of milk fever. It is also apparent that the serum calcium-magnesium relationship is more important in producing the symptoms of milk fever than is serum phosphorus.

Figure 1 shows the changes in the serum calcium and serum phosphorus of the blood of normal and milk fever Jersey cows at various times before and after parturition. Fifteen minutes after treatment with calcium gluconate (500 cc.) the serum calcium was found to be 17.1 mg. per cent which declined rapidly to near the normal level. If the fall in serum calcium continued too long a recurrent attack of milk fever ensued. Both control and yeast-fed cows are included in this figure, since, as shown in tables 1 and 2, yeast feeding at this level did not affect the blood levels of calcium and phosphorus.

The changes in the serum calcium, phosphorus, and magnesium of control and milk fever Jersey cows before and after parturition are shown in figure 2. Marked decreases in serum calcium and phosphorus occur prior to the onset of milk fever accompanied by a concurrent increase in serum magnesium.

The phosphatase content of serum was variable but it appears, as shown in table 3, that in Jersey cows especially, the phosphatase was lower at freshening than before or after freshening and that the lowest values were obtained in milk fever. This may be of significance in view of the low phosphorus content of blood serum during milk fever.

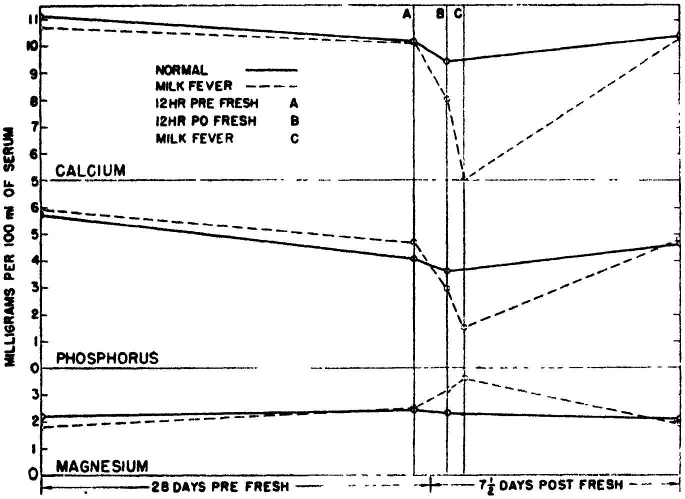

FIG. 2. Changes in the serum calcium, phosphorus, and magnesium of the blood before and after parturition of 25 control Jerseys fresh normally and 13 control Jerseys that developed milk fever.

While the serum protein values shown in table 5 were all within the normal range, a slight decrease was observed after parturition in both normal and milk fever cows. The lowest values obtained were in samples taken during the milk fever attack. It is not known whether or not this is of any importance in connection with milk fever, but it does indicate that cows in beginning lactation and in milk fever may be somewhat deficient in body protein, since low serum protein does not usually occur until after a pronounced loss of body protein. The lowering of serum protein at parturition and in milk fever is probably due, at least in part, to the formation of colostrum which is extremely high in protein, as indicated in table 9.

No marked changes at parturition were found in the plasma ascorbic acid content of the blood; however, plasma carotene and vitamin A were greatly reduced at this time. The low values obtained during milk fever indicate that blood vitamin A and carotene values either continue to fall after the 12-hour post-freshening sample or that in milk fever the decrease is delayed. The latter concept supports the observation that in many milk fever cows the udder does not fill before parturition. Colostrum is extremely rich in these constituents, as shown in table 9. Judging from the relative decreases in the blood level of vitamin D and vitamin A it is doubtful whether or not there is relatively as high a concentration of vitamin D in colostrum as there is of vitamin A. This is also suggested by the work of Henry and Kon (18).

Table 9 shows the fat content of colostrum to be somewhat variable but on the average it contains less fat than typical Jersey milk. No difference in specific gravity, total solids, or ash content was found between colostrum from normal cows and that of milk fever cows. All three of these factors were extremely high in concentration in colostrum as compared to normal milk.

While no marked difference was observed in the composition of the colostrum of normal and milk fever cows, it is evident from the high concentration of vitamin A, carotene, protein, ash, and total solids that the formation of colostrum constitutes a sudden, heavy drain on the body reserves of the parturient cow. This is further substantiated by the corresponding fall in the blood level of these constituents.

Whether or not a cow develops milk fever seems to be determined by the success or failure of the mineral regulatory mechanism to meet the sudden demands of beginning lactation and at the same time maintain the necessary blood levels of calcium, phosphorus, and magnesium.

As indicated in tables 1, 4, and 8 the data concerning blood vitamin D were exceedingly variable. However, a few general statements can be made regarding the results. Although there was a tendency for the blood vitamin D to decrease at parturition, the decrease was not so marked as that of vitamin A and carotene. No clear-cut breed difference is noted; however, the Jerseys seemed to have a lower average blood vitamin D level than did the other three breeds. Some of the lowest values for blood vitamin D were obtained during the milk fever attack, but there was no correlation between the incidence of milk fever and the vitamin D content of the blood, since milk fever occurred in some cases even though the blood vitamin D content was twice normal. The data also show that an approximate twofold increase in the blood vitamin D, as a result of feeding 1 million units of vitamin D daily, did not affect the calcium and phosphorus level of blood serum.

When 2 million units of vitamin D were fed daily the vitamin D content of the blood was found to be approximately four times that of the controls. At this level of vitamin D feeding a slight increase in serum calcium and phosphorus was observed. It would seem, therefore, that before any marked effect on the serum calcium and phosphorus can be expected, more than 2 million units of vitamin D must be fed daily. It appears, then, that the mere raising of blood vitamin D above the normal level will not prevent milk fever from occurring. It is possible that only when the blood vitamin D concentration reaches a level sufficient to elicit an increase in the blood calcium and phosphorus, a decrease in milk fever incidence might be expected. More work needs to be done before this question can be answered.

SUMMARY AND CONCLUSIONS

1. Determinations were made on the blood serum for calcium, phosphorus, magnesium, and phosphatase, and on whole dried blood for vitamin D content, of control cows and cows fed 1 and 2 million units of vitamin D daily for four weeks before and one week following parturition, fresh normally and with milk fever. Cows of the Jersey, Holstein, Ayrshire, and Guernsey breeds were included in the study.

2. Data are presented regarding the serum protein, plasma carotene, vitamin A, and ascorbic acid of Jersey cows that freshened normally and with milk fever.

3. A study was made of the specific gravity, total solids, ash, fat, total protein, carotene, and vitamin A in the colostrum of normal and milk fever cows fed various amounts of vitamin D.

4. No effect on the serum calcium, phosphorus, magnesium, or phosphatase was observed when 1 million units of vitamin D in the form of irradiated dry yeast were fed daily for four weeks previous to freshening and for one week thereafter. When 2 million units of vitamin D were fed, a slight increase in both serum calcium and phosphorus was noted. This was accompanied by a slight decrease in serum magnesium. It was found that both serum calcium and phosphorus decrease markedly during a few hours prior to the milk fever attack, the lowest level being reached after the symptoms appear. A concurrent increase in the serum magnesium usually occurs.

The level of the blood vitamin D was found to be approximately double the normal level when 1 million units of vitamin D were fed and about four times the normal level when 2 million units of vitamin D were fed.

Before increases in the serum calcium and phosphorus level of the blood can be obtained, sufficient to lower milk fever incidence, apparently more than 2 million units of vitamin D must be fed daily before freshening. Experiments are now in progress to determine the effect of feeding larger amounts of vitamin D on the blood picture and the incidence of milk fever.

It is of interest that some of the lowest blood vitamin D values obtained occurred in control cows during the milk fever attack; however, when vitamin D was fed milk fever occurred even though the blood level of vitamin D was relatively high. The decrease in the blood vitamin D of control cows at parturition is considered to be associated with the production of colostrum.

5. The decreases observed in serum protein and plasma vitamin A and carotene at parturition are considered to be associated with the production

of colostrum which is particularly rich in these constituents. No significant change in the level of plasma ascorbic acid was noted at parturition.

6. No significant difference was noted in the composition of colostrum between cows that freshened normally and those which developed milk fever.

7. The blood serum calcium-magnesium relationship is discussed in regard to its possible effect on the symptomatology of milk fever.

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THE PANTOTHENIC ACID, NIACIN, AND BIOTIN CONTENT OF COMMERCIAL AND EXPERIMENTAL MILKS*

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The pantothenic acid content of milk has been reported by various investigators (1, 3, 4, 11, 13) to range from 1.3 to 4.6 μg. per ml. Williams *et al.* (13) found the range to be 1.7 to 4.6 with an average value of 2.9 μg. per ml. for 30 samples. Fresh milk analyzed by Hodson (3) contained from 1.9 to 4.2 with an average of 3.1 μg. per ml.

Early methods for the determination of niacin were of a chemical nature and proved to be unreliable and figures ranging from 0.6 to 8.2 μg. per ml. have been reported (2, 7). With a reliable microbiological assay Williams *et al.* (13) obtained minimum and maximum values of 0.19 and 1.2 μg. per ml., respectively. Values given by other workers (3, 10, 12) fall within this range. Hodson (3) analyzed 31 samples collected during the month of May and found an average of 0.91 μg. per ml.

Reports on the biotin content of milk (3, 5, 8, 13) vary from 12.5 to 110 mμg. per ml. Hodson (3) reported minimum and maximum values of 32 and 84 and an average of 47 mμg. per ml.

All of these reports deal with the analysis of one sample or of samples collected for a short period of time or of milk collected at irregular intervals. It seemed desirable therefore to analyze samples of market milk collected throughout the year. At the same time an experiment was set up to determine if there is a relation between the type of silage consumed by cows and the content of these vitamins in their milk.

EXPERIMENTAL

Milks. The samples of commercial milk were either purchased in the open market or delivered by the distributor. Three distributors supply the city of Madison. These milks are classified as market and Guernsey milk and represent a daily distribution of about 110,000 lbs. of milk.

In February 1943, a number of milk samples were obtained from individual farms in the vicinity of Madison which were included in a grass silage program sponsored by the College of Agriculture. Table 2 presents the analytical results as well as the forage fed to these herds. Ordinary farm rations were being fed at the time the samples were taken.

The experimental milks were produced by animals on silage experiments at the University farm. In the 1940–41 and in the 1941–42 trials at the

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University farm, each lot contained five cows while in the 1942–43 experiments, each group consisted of nine cows. The animals were fed a ration consisting of 40 to 50 lbs. silage, 5 to 10 lbs. good hay and 3 to 12 lbs. grain per day. Variations in the ration were made according to the milk production of the animal.

Analytical methods. Aliquots of the milks were diluted with distilled water to the proper level and analyzed without further treatment. All milks were frozen after the aliquots for dilution had been taken so that repeat analyses could be run if the analytical results seemed doubtful. Recoveries of added pantothenic acid, niacin and biotin were run with all assays. If a recovery did not fall within ± 10 per cent, the determination was repeated. Repeat analyses on reference materials were run regularly as an additional check on the methods.

Pantothenic acid was determined by the method of Strong *et al.* (11). Williams *et al.* (13) reported higher values for samples of skim milk digested with takadiastase and papain than for the untreated samples. Digestion with 2 per cent of an equal mixture of takadiastase and papain was tried on our milks but no effect on the results was obtained.

The Snell and Wright method (10) for the determination of niacin with HCl in place of H_2SO_4 for hydrolysis of casein was used.

The *Lactobacillus casei* method described by Shull *et al.* (8) and modified by Shull and Peterson (9) was used for the determination of biotin. Tests in which samples were digested with a mixture of takadiastase and papain or hydrolyzed with 4.0 N HCl for 2 hours were run to determine whether biotin was present in bound form. The results obtained showed that these treatments were not necessary.

RESULTS AND DISCUSSION

Pantothenic acid. Table 1 summarizes the analyses of the commercial milks. The pantothenic acid content of the samples ranged from 2.7 to 4.5 μg. per ml. for the individual samples. Only one sample contained more than 3.9 μg. per ml. pantothenic acid. The average values for market and Guernsey were 3.4 and 3.2 μg. per ml. respectively. The pantothenic acid content did not vary greatly with seasonal changes. In 1940 and 1941, 29 other samples of commercial milk were analyzed by G. P. Bahler of this department and were found to range from 2.7 to 5.9 with an average of 4.2 μg. per ml.

Individual farm milks (table 2) were somewhat higher in pantothenic acid than commercial milks but approximately the same as the experimental milks.

The values for experimental milks in table 3 are averages of from 2 to 4 samples. The range of pantothenic acid from the low to the high samples of milk in a given feeding trial (table 3) was in most cases about 20 per

TABLE 1

B vitamin survey of commercial milks

Date	Pantothenic acid		Niacin		Biotin	
	Market*	Guernsey†	Market*	Guernsey†	Market*	Guernsey†
	µg./ml.		*µg./ml.*		*mµg./ml.*	
August 4, 1942	3.2	3.1	0.82	0.83	29	24
September 10, 1942	3.1	3.0	0.77	0.79	30	27
January 24, 1943	3.7	3.7	0.75	0.72	31	32
April 16, 1943	3.5	3.4	0.71	0.74	32	29
June 9, 1943	3.3	3.0	0.95	0.88	21	21
Total number of samples	29	15	29	15	29	15
Range of samples	2.7–4.5	2.7–3.8	0.64–1.04	0.63–1.08	18–37	17–38
Average	3.4	3.2	0.80	0.79	29	27

* The figures are the average for 6 samples at each date except the August 4, 1942, values when only 5 samples were run.

† The figures are the average for 3 samples.

cent. The commercial milks showed a variation of about 50 per cent. These variations agree with those reported by Williams *et al.* (13) and Hodson (3). The pantothenic acid values were higher for the winter and spring samples of 1940 and 1941 than for those for the following winter and spring, 1942–43. No correlation can be made between the roughage fed and the variation in the pantothenic acid content of these experimental milks.

Niacin. The niacin content of commercial milk (table 1) ranges from 0.63 to 1.08 µg. per ml. for individual samples of market and Guernsey milk. The averages for market milk and Guernsey milk were 0.80 and 0.79 µg. per ml., respectively. The niacin content increased slightly during the summer months. The values for commercial milks were generally higher than those for the individual farm milks and experimental milks. The average niacin content of experimental milks was 0.63 µg. per ml. A 10 per cent variation among samples of experimental milks and about 50 per cent among the commercial samples was found in most cases. Similar variations are reported by Williams *et al.* (13) and Hodson (3).

TABLE 2

Analyses of individual farm milks for pantothenic acid, nicotinic acid and biotin

Farm	No. of samples	Silage	Pantothenic acid	Niacin	Biotin
			µg./ml.	*µg./ml.*	*mµg./ml.*
1	1	Alfalfa and corn-cob meal silage	4.0	0.72	49
2	1	Sweet clover and corn-cob meal silage	4.6	0.75	35
3	1	Alfalfa silage—no preservative	3.2		62
4	1	Corn silage	4.4	0.52	28
5	2	Alfalfa and corn-cob meal silage	6.0	0.52	38

TABLE 3

Analysis of experimental milks from the University herd

Month and year		No. of cows	No. of samples	Roughage	Pantothenic acid	Niacin	Biotin
					μg./ml.	μg./ml.	mμg./ml.
Nov.–Feb.	1940–41	5	4	Oats-peas-H_3PO_4 silage	4.4 ± 0.3		40*
Nov.–Feb.	1940–41	5	4	Soybean-sudan-H_3PO_4 silage	5.0 ± 0.5		59*
Nov.–Feb.	1940–41	5	4	Alfalfa-molasses silage	4.3 ± 0.5		50*
Feb.–Mar.	1942	5	3	Soybean (2)–sorghum (1) silage	4.4 ± 0.4	0.68 ± 0.03	47 ± 4
April	1942	5	3	Soybean (1)–sorghum (1) silage	5.5 ± 0.5	0.61† ± 0.03	45 ± 3
Feb.–Mar.	1942	5	3	Alfalfa–10–20 lb. conc. whey/ton silage	4.3 ± 0.1	0.62 ± 0.0	53 ± 3
Feb.	1942	5	1	Alfalfa—200 lb. corn-cobmeal/ton silage	3.6	0.59	41
Mar.	1942	5	2	Alfalfa silage—no preservative	4.1 ± 0.0	0.66 ± 0.02	55 ± 3
Mar.–April	1942	6	2	Corn silage	4.1 ± 0.1	0.74*	20 ± 1
July–Sept.	1942	20	3	Pasture	3.3 ± 0.3	0.70 ± 0.09	27 ± 7
Nov.	1942	18	1	Corn silage	3.4	0.69	28
Jan.	1943	18	1	Corn silage	3.2	0.76	45
Mar.–April	1943	9	2	Alfalfa silage—200 lb. corn-cobmeal/ton	4.4 ± 0.3	0.59 ± 0.04	42 ± 4
Mar.–April	1943	9	2	Alfalfa silage—forage wilted	3.5 ± 0.9	0.37 ± 0.06	38 ± 4
June	1943	18	1	Corn silage	3.4	0.53	40

* Analysis on one sample.
† Analysis on two samples.

Biotin. The biotin content of commercial milk for the period beginning August 4, 1942, and ending June 9, 1943, varied from 17 to 38 mμg. per ml. for individual samples of market and Guernsey milk (table 1). The average values for these two classes of milk were 29 mμg. per ml. for market milk and 27 mμg. per ml. for Guernsey milk. There was a notable decline in the biotin content during the month of June when the cows were pasture fed. The milks from the individual farms (table 2) ranged from 28 to 62 mμg. per ml. of milk. This wide range may be related to the type and the quality of silage fed. High quality silage was fed on farms 1, 2, 3, and 5 and very poor quality of corn and grass silages were fed on farm 4.

The experimental milk (table 3) shows a marked variation in biotin content. A minimum of 20 mμg. per ml. and a maximum of 59 mμg. per ml. were obtained. Grass silage milks generally were higher in biotin than corn silage or pasture milk, however, high biotin values (January 1943) have been obtained when corn silage was the roughage fed. A comparison of the data from tables 1 and 3 shows that there was a decrease in the biotin content of milks produced on pasture. A part of the University herd consisting of 18 cows which was still on winter feed in June, showed no decline in the biotin content of the milk. A possible explanation of this decline may be that because of changes in the bacterial flora, the synthesis of biotin in the rumen on pasture feed does not keep pace with the increased milk flow. Lardinois *et al.* (6) have shown that an increase in the synthesis of vitamins of the B complex in the rumen of cows can be brought about by the addition of urea as a source of nitrogen in the ration. Similarly, other feeding changes might influence the biotin content of the rumen and consequently the biotin content of the milk.

SUMMARY

The pantothenic acid content of commercial market and Guernsey milks for the year 1942–43 averaged 3.4 and 3.2 μg. per ml., respectively. Analyses of 29 samples of commercial milk the preceding year averaged 4.2 μg. per ml. The niacin content of commercial milk averaged 0.80 μg. per ml. Seasonal and feeding changes did not affect the amount of these two vitamins.

Milks analyzed for biotin were found to contain from 17 to 62 mμg. biotin per ml. The average values for market and Guernsey milks were 29 and 27 mμg. per ml., respectively. The biotin content of milk showed seasonal variations which were the result of feeding changes. The type of roughage fed influenced the biotin content of milk.

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They are indebted to I. W. Rupel, Dairy Husbandry Department, and G. Bohstedt, Animal Husbandry Department, for cooperation in respect to the silages and milks of table 3. These silages were put up and fed to determine their value in maintaining the carotene and vitamin A content of winter milk. A report on this work will be given in another publication.

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THE CAROTENE AND VITAMIN A CONTENTS OF WISCONSIN CHEESE*

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INTRODUCTION

In an earlier paper from this laboratory (5) it was reported that several hundred samples of Wisconsin market milk averaged 32 I.U. of vitamin A potency per gram of butter fat for winter (December–April) milk and 55 I.U. for summer (May–November) milk. A similar survey (1) of the market butter produced in this state showed a potency of 27 I.U. per gram of butter fat for the winter product and 49 I.U. for the summer butter. A recent report (11) on the situation in the whole United States gave the average potency as 11,160 I.U. per lb. for winter and 17,955 I.U. per lb. for summer butter. Assuming 80 per cent butter fat, these figures correspond to 30 I.U. and 49 I.U. per gram of butter fat for the respective seasons. If the United States figures for butter fat are applied to cheese on the basis of 150 grams of fat per lb. of cheese and assuming no loss of potency in processing, the vitamin A potency should be about 4,500 I.U. per lb. for the winter cheese and 7,400 for the summer product. These calculations are for whole milk cheese such as cheddar, in which the butter fat is about 31 to 33 per cent. The calculated values are considerably below many of the figures found in the literature. Hathaway and Davis (6) reported about 7,000–10,900 I.U. per lb. for cheddar, 18,200 I.U. per lb. for Roquefort, and as high as 19,000 I.U. per lb. for pimento cream cheese. The first two types contained approximately 30 per cent fat while the pimento cream type contained only 18.4 per cent fat. Coward and Morgan (3) reported a sample of English cheddar cheese to have almost 25,000 I.U. per lb. Other values found in the literature are as follows: 3,400 I.U. per lb. for cheddar cheese, Davies and Moore (4), 11,000 I.U. per lb. for Roquefort cheese, Todhunter *et al.* (10), and about 9,000 I.U. per lb. for Porto Rican cheese, Cook and Axtmeyer (2). Most of these data have been obtained by the rat assay method, and in some cases undoubtedly reflect other factors than the vitamin A potency. Since the available data do not show clearly the seasonal variations in the product, it seemed desirable to make a survey of the vitamin A potency of the principal types of cheese in relation to the season of production and also with respect to the grade of the product.

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EXPERIMENTAL

Collection of samples. Cheddar and Swiss cheeses comprised the bulk of the samples, but a few other types were also examined. The collections were made monthly by the district inspectors of the State Department of Agriculture. Over five hundred samples in all were obtained during the period from the early summer of 1944 to the same season in 1945. Seven districts representing the principal cheese-producing areas were included. The samples were shipped in two-ounce screw-lid jars and kept in the cold room at below 0° C. until analyzed.

Analytical method for carotene and vitamin A. The procedure used for the determination of the carotene and vitamin A contents of cheese has been described in another paper (7). The amount of carotene was measured by its absorption of light at 440 mμ and vitamin A was determined by its absorption at 620 mμ after treatment with antimony trichloride in chloroform.

RESULTS AND DISCUSSION

Seasonal variation of the vitamin A potency of cheese. The samples were collected and analyzed monthly but the data can be adequately summarized by two- or three-month periods covering pasture and dry-feed rations. The data for cheddar cheese are given in table 1. The minimum values for carotene and vitamin A occurred in the March–April period when the cows had been off pasture for the longest time and spring pasturing had not yet begun. The values were 0.78 μg. per gram for carotene and 1.66 μg. per gram for vitamin A. The maximum values occurred in the September–October period with figures of 2.97 μg. per gram for carotene and 3.11 μg. per gram for vitamin A. Calculated as I.U. per gram on the basis that 0.6 μg. carotene and 0.25 μg. vitamin A are each equal to one I.U., the minimum and maximum values were 7.94 I.U. per gram and 17.39 I.U. per gram, respectively. This corresponds to values of about 3600 I.U. per lb. and 7900 I.U. per lb., respectively.

TABLE 1

Vitamin A potency of cheddar cheese

Period	No. samples	Carotene		Vitamin A		Total potency*
		μg./g.		*μg./g.*		*I.U./g.*
		range	ave.	range	ave.	
Jan.–Feb.	84	(0.58–1.38)	0.93	(1.35–2.03)	1.80	8.75
Mar.–April	96	(0.40–1.33)	0.78	(1.11–2.27)	1.66	7.94
May–June	75	(0.75–4.45)	2.34	(1.40–3.20)	2.50	13.90
July–Aug.	37	(1.67–3.48)	2.46	(1.90–3.42)	2.79	15.26
Sept.–Oct.	78	(2.13–3.35)	2.97	(2.80–3.72)	3.11	17.39
Nov.–Dec.	13	(1.15–1.80)	1.44	(1.80–2.60)	2.02	10.48

* Based on the assumption that 0.6 μg. carotene and 0.25 μg. vitamin A are each equal to one International Unit.

TABLE 2

Vitamin A potency of Swiss cheese

Period	No. samples	Carotene		Vitamin A		Total potency*
		µg./g.		µg./g.		I.U./g.
		range	ave.	range	ave.	
Feb.-April	18	(0.35-0.73)	0.56	(1.27-2.10)	1.70	7.73
May-July	46	(1.08-2.20)	1.69	(1.81-2.75)	2.35	12.21
Aug.-Oct.	37	(1.63-1.95)	1.75	(2.78-3.34)	2.94	14.67
Nov.-Jan.	27	(0.50-1.73)	0.99	(1.60-2.85)	1.97	9.53

* Based on the assumption that 0.6 µg. carotene and 0.25 µg. vitamin A are each equal to one International Unit.

The data for Swiss cheese are summarized by three-month periods because of a smaller number of samples (table 2). Here the same trend as with cheddar cheese was observed. The maximum values occurred in the August–October period with 14.67 I.U. per gram (6600 I.U. per lb.), and the minimum occurred in the February–April period with 7.73 I.U. per gram (3500 I.U. per lb.). The values for Swiss cheese were generally lower than in cheddar samples. The vitamin content, while not exactly proportional, roughly paralleled the fat content of the two types of cheese. Fat determinations showed about 26 per cent fat in the Swiss type and about 33 per cent fat in the cheddar type of cheese.

Grade of cheese and vitamin A potency. In order to determine whether there was any relation between grade and vitamin A potency, the data were tabulated according to the various grades reported by the inspectors for the cheddar and the Swiss samples (table 3). Though variations occurred among the different grades, there was no correspondence between grade and vitamin A potency. In the instances where there seemed to be a difference between grades the difference can probably be attributed to an insufficient

TABLE 3

Variations of the carotene and vitamin A contents of cheddar and Swiss cheese with season and grade

	Feb.-April		May-July		Aug.-Oct.		Nov.-Dec.	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
	µg./g.	µg./g.	µg./g.	µg./g.	µg./g.	µg./g.	µg./g.	µg./g.
Cheddar grades								
State	0.77	1.64	2.10	2.43	3.02	3.07	1.09	1.88
Junior	0.78	1.68	2.50	2.61	3.02	3.13	1.00	1.87
Under	0.75	1.70	2.50	2.63	2.70	3.01	0.94	1.82
Swiss grades								
A	0.51	1.47	1.77	2.38	1.65	2.84	0.76	1.80
B	0.50	1.69	1.81	2.28	1.70	3.09	1.35	2.45
C	0.53	1.70	1.88	2.43	1.80	3.01	0.97	1.86
No. 3	0.66	1.75	1.69	2.35	1.80	2.92	0.96	1.92
Grinder	0.58	1.81	1.66	2.47	1.71	2.85	1.16	2.13

number of samples. Cheese grading clearly does not distinguish differences in vitamin A potency. Because of the importance of vitamin A in nutrition, it would seem reasonable to include the vitamin A potency of the sample in establishing its grade.

Miscellaneous types of cheese. A few samples of cheese other than cheddar and Swiss were analyzed. Brick, Muenster, Limburger, and Colby all showed no significant difference from cheddar cheese of the same season in their vitamin A potency. Since about 85 per cent of the carotene and vitamin A content of the milk are found in the cheese, and curing and storage do not change the values (7), the vitamin A potency of cheese corresponds to the potency of the milk and this in turn reflects the carotene content of the ration. The vitamin A potency of milk, butter, and cheese produced during the winter months could probably be increased at least 50 per cent by making and feeding good quality grass and corn silages (8, 9).

SUMMARY

Over five hundred samples of cheese, chiefly of the cheddar and Swiss types, were collected and analyzed for their carotene and vitamin A contents.

The maximum vitamin A potency of cheddar cheese occurred in those samples made in the September–October period, 17.39 I.U. per gram (7900 I.U. per lb.); the minimum occurred in the March–April period, 7.94 I.U. per gram (3600 I.U. per lb.). The values for Swiss cheese were: August–October 14.67 I.U. per gram (6600 I.U. per lb.) and February–April 7.73 I.U. per gram (3500 I.U. per lb.). A few samples of cheese other than cheddar or Swiss were analyzed. Brick, Muenster, Limburger and Colby all showed no significant difference from cheddar cheese of the same season in vitamin A potency.

No correspondence between vitamin A potency and grade of cheese was observed among the samples.

The vitamin A potency of cheese corresponds to that of the milk from which it was made and this in turn reflects the carotene content of the ration.

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SOME OBSERVATIONS ON "QUALITY" IN HAYS*

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Much has been said and written about quality in roughage, but nobody seems to be able to define the word or to say what should be looked for in any analysis to determine quality. The ultimate verdict must of course be given by the animal which eats the roughage. If it is unpalatable, or (in the case of a milking cow) if it fails to maintain milk production satisfactorily, then we know that its quality is inferior. When that phase of the situation has been reached, however, it is often too late to do much about it. Cannot some other criterion be established which would enable the evaluation of quality before roughage is fed out, or which might explain the great variations in feeding value found in different lots of hay, especially the differences which are noted from season to season? The work here reported, although admittedly fragmentary and of a preliminary nature, constitutes a modest step toward an answer to this very important question.

The growing season of 1945 in southern New England was characterized by excessive rainfall and less than the average amount of sunshine. Although pasture and hay crops were well above average in yield, the pastures did not stimulate milk production as much as it was expected they might, and curing weather for the hay crop was so poor that much of it was stored in poor condition. Soon after the barn feeding season started in the autumn, some farmers began to complain that their cows were not holding up in production as they should, despite heavy grain feeding. By the end of the year the condition was noted in various parts of the state and some of the farmers turned to the State College for assistance. One prominent Jersey breeder submitted samples of four lots of hay grown on his farm with the information that his cows varied so in their response to them that he was curious as to what might be the cause. Questions as to his other feeding and management practice revealed the fact that the different lots of hay were the only known variable.

As a first step in the search for possible clues, a routine fodder analysis was made of all four samples. Results of this showed that in comparison with average analyses of the kinds of hay in question, protein, fat, and ash were about normal, but that fiber was invariably above average, as much as 6 to 8 per cent in some of the samples, while nitrogen-free extract was correspondingly lower. Considerable differences were noted also in the individual samples in this respect.

The question at once arose as to what might be lacking in the nitrogen-

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TABLE 1

Results of analyses of hays

Crop year	Protein	Ether extract	Fiber	N-free extract	Ash	Total sugars	Carotene	Quality[a]
Mixed grass hay—largely timothy—1st cutting								
1943	5.0	1.6	38.9	51.9	2.6	6.2	3.4	Poor*
	6.1	1.6	37.0	52.2	3.1	8.9	1.2	Fair*
	10.0	1.6	37.5	46.6	4.2	3.8	1.8	Poor*
	6.7	1.8	37.9	50.4	3.2	6.8	1.9	Fair*
Average, 1943	7.0	1.6	37.8	50.3	3.3	6.4	2.1	
1944	8.0	1.8	36.2	50.2	3.7	3.3	N.D.	Good*
	9.4	2.2	38.7	44.6	5.1	4.2	5.7	Fair*
Average, 1944	8.7	2.0	37.5	47.4	4.4	3.8		
1945	8.8	1.7	40.0	43.5	6.0	1.3	3.0	Poor
	5.7	1.4	42.0	46.2	4.7	3.0	1.6	Poor
	5.7	2.0	36.3	51.1	4.8	4.2	4.0	Very good
Average, 1945	6.7	1.7	39.4	46.9	5.2	2.8	2.9	
For comparison	7.2	2.6	33.3	50.4	6.3	No data	3.0[b]	
Mixed grass hay—2nd cutting								
1944	11.1	2.0	30.2	51.3	5.5	4.4	N.D.	Good*
For comparison	13.8	3.7	27.9	46.9	7.8	No data		
Mixed grass and legume hay—1st cutting								
1944	11.1	1.4	40.2	40.3	7.0	0.9	1.6	Fair*
1945	9.5	2.1	36.5	45.5	6.4	3.0	3.2	Good*
	13.2	1.9	37.1	41.4	6.4	5.0	6.6	Good
	8.2	1.6	35.7	48.5	6.0	5.8	3.2	Good
	11.4	2.0	37.5	43.2	5.8	3.9	4.8	Fair*
	7.5	2.0	37.8	46.9	5.8	5.8	6.4	Good
	9.2	1.9	38.6	43.7	6.6	3.1	2.9	Fair*
Average, 1945	9.8	1.9	37.2	44.9	6.2	4.4	4.5	
For comparison	9.8	2.3	34.2	46.6	6.0	No data	12.0	
Mixed grass and legume hay—2nd cutting								
1943	14.1	2.2	28.5	49.6	5.6	8.4	30.0	Excellent*
1945	15.4	3.5	36.0	36.7	8.4	1.6	15.4	Good
	19.1	2.8	32.7	36.7	8.7	2.7	20.3	Good
Average, 1945	17.3	3.2	34.4	36.7	8.6	2.2	17.9	
For comparison	13.3	3.3	31.3	43.4	6.5	No data	26.0	
Mixed grass hay—native, fine leaved grasses (cut in August)								
1945	8.2	2.5	35.1	49.5	4.7	4.6	4.4	Fair
	9.8	3.2	35.9	45.3	5.9	4.4	20.7	Good
Average	9.0	2.9	35.5	47.4	5.3	4.5	Not averaged	......
For comparison	7.9	2.8	34.7	48.4	6.2	No data	No data	..

TABLE 1 (*Continued*)

Red clover hay								
1943	16.0	2.6	29.8	45.3	6.4	5.2	18.3	Good*
1945	17.1	2.3	34.2	37.9	8.5	2.4	8.9	Good*
For comparison	13.4	2.9	31.0	45.5	7.3	No data	7.6	
Reeds canary grass								
1945	8.0	1.5	41.4	42.9	6.3	1.3	1.4	Poor
For comparison	8.3	2.6	32.0	48.9	8.1	No data	No data	. .

All values are expressed on a dry matter basis and all except carotene are percentages; carotene is expressed as thousands of International units of vitamin A equivalent per pound.

[a] Quality was determined on either or both of two bases as indicated: 1—the farmer's opinion based on milk production when the hay was fed; 2—appearance of the sample when received as judged by leafiness, color, aroma, and freedom from mold, weeds and other trash. In those cases where the farmer did not evaluate the hay, the second basis of grading had to be relied on entirely. Samples so graded are marked with an asterisk. Where the farmer expressed an opinion, his estimate was given more consideration than ours in determining quality if the two did not agree. For obvious reasons, the results of analysis were not a factor in the decisions.

[b] Average carotene values are not available in Morrison's tables. Those reported for comparison have been compiled from several sources of rather meager data. They may not be strictly comparable with the values determined in the 1943, '44, and '45 samples, due to improvement in recent years in methods for carotene determination. The improved methods now in use generally give lower carotene values, the average being about a third less.

N.D. = not determined.

free extract, and the answer seemed obvious at once—soluble carbohydrates. It was therefore decided to determine total sugars not only in these four samples but in as many additional samples of the 1945 hay crop as might be readily obtainable. Twenty-one other hay samples were secured, eleven of them from the 1945 crop, making a total of fifteen for 1945, four from the 1944 crop, and six from the 1943 crop. The samples came from five different localities well scattered over the state.

Total sugars were determined by the procedure outlined in Methods of Analysis of the A.O.A.C. (1), using the method of Lane and Eynon. Carotene was also determined using the method of Wall and Kelley (4), and routine fodder analyses were made on all samples. Table 1 shows the results classified by seasons according to kind of hay, together with average analyses as given by Morrison (2) for comparison.

Comparing the 1945 samples with those for 1943 and 1944, it is seen that protein alone in most cases was average or above. Ether extract, total ash and carotene were generally below average. With one exception fiber was higher in the 1945 samples, and wherever it was higher, nitrogen-free extract was lower. Wherever nitrogen-free extract was relatively low, total sugars were also relatively low. Some of the differences in content of total sugar from year to year are marked.

There is a rather close correlation between quality as determined by feeding value and appearance, and relative amounts of desirable and undesirable components as shown by analysis. In other words, the better quality hays in general were higher in such constituents as protein, carotene, and sugar, and lower in crude fiber than those hays which were graded as of fair or poor quality, and this holds true regardless of the kind of hay. It seems therefore that the complaints by many farmers as to poor feeding value of the 1945 hay crop, are corroborated to a considerable degree by these results.[1]

TABLE 2

Records of rainfall and sunshine at Amherst, Massachusetts, for the growing season of 1945 in comparison with 1943 and 1944, and the 50-year average

Month	1943	1944	1945	50-year average (1889–1938 incl.)	Percentage which 1945 record is of 50-year average
			Rainfall in inches		
April	3.66	3.66	5.43	3.35	162.1
May	5.62	1.35	6.45	3.60	179.2
June	2.38	4.70	7.67	3.75	204.5
July	6.18	3.88	7.36	4.10	179.5
August	2.49	4.33	2.79	4.08	68.4
September	2.40	5.31	3.57	4.24	84.2
Total for the growing season	22.73	23.23	33.27	23.12	
Percentage of the 50-year average	98.3	100.5	143.9		
			Sunshine—per cent of possible hours of bright sunshine		
April	61.4	54.7	64.2	54.7	117.4
May	60.5	77.3	41.9	55.6	75.4
June	77.9	61.1	53.0	54.0	98.1
July	74.0	72.5	59.5	58.0	102.6
August	71.5	78.7	61.3	55.2	111.1
September	61.1	55.5	46.6	54.9	84.9
Average	67.7	66.6	54.4	55.4	
Percentage of the 50-year average	122.2	120.2	98.2		
			Average monthly temperatures—°F.		
April	41.0	42.9	51.8	45.7	113.3
May	57.8	63.4	54.1	57.1	94.7
June	71.1	66.3	66.1	65.7	100.6
July	71.8	72.9	70.6	70.8	99.7
August	68.9	72.4	68.3	68.6	99.6
September	61.1	63.0	64.6	61.7	104.7
Average	62.0	63.5	62.6	61.6	
Percentage of the 50-year average	100.6	103.1	101.6		

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[1] In the absence of actual feeding trials, certain assumptions as to feeding value of these hays have been made. These assumptions are believed to be valid in the light of our general knowledge of the relationship between feeding value and composition; also they are confirmed somewhat by the opinions of the men who fed some of the lots of hay which the samples represent.

In searching for a reason for the relatively poor quality of the 1945 crop, weather conditions come to mind at once. As already noted, 1945 was an unusaully rainy season in this region, especially during the early summer. How it compared with 1943 and 1944, and how far it departed from the 50-year average at Amherst, Massachusetts, are shown in table 2.

These data, although for only one locality, are reasonably representative of conditions throughout Massachusetts in 1945. The records which stand out as unusual are the rainfall in May, June, and July and the relative lack of sunshine in May. May is the month when our first crop of hay makes most of its growth; June and July are the months when normally most of it is harvested. Since direct sunshine and photosynthetic activity are closely correlated, under definite conditions some relationship might be expected between unusually cloudy weather in May and the relatively low levels of total sugar in the 1945 samples. Probably of greater importance, however, than lack of sunshine in lowering the sugar level in the hay was the extremely unfavorable curing weather in June and July. This is evidenced by an amount of rainfall more than double the 50-year average for June, and nearly double for July. As a result, much hay was stored improperly cured, with consequent excessive "sweating," a biochemical process known to proceed at the expense of soluble organic compounds.

Good illustrations of this are the two samples of 2nd cutting mixed grass and legume hay (1945 crop). Although of good quality in other respects, these samples were low in sugar and quite high in fiber for 2nd crop hay. Examination of the samples when received revealed considerable mold and musty odor, conditions which develop when hay is stored too damp, either from natural moisture or from rain.

Average seasonal temperatures in 1945 did not deviate markedly from the 50-year average. In the month of May, however, the average temperature was considerably below the 50-year figure (3° F.), and rainfall was abundant; May is the month when the first crop of hay in this region makes most of its growth. In general, temperature and water content of the plant

TABLE 3

Composition of hays cured by different methods

Method of curing	Protein	Ether extract	Crude fiber	N-free extract	Total ash	Total sugars	Carotene	Quality
Mixed hay—largely timothy—1st cutting								
Field-cured:								
Loose	5.7	2.0	36.3	51.1	4.8	4.2	4.0	Very good
Baled	8.8	1.7	40.0	43.5	6.0	1.3	3.0	Poor
Mixed hay—grass and legumes—1st cutting								
Field-cured:								
Loose	9.0	1.9	36.9	45.9	6.2	4.0	3.1	Good
Baled	13.2	1.9	37.1	41.4	6.4	5.0	6.6	Good
Blower-dried:								
Loose	7.5	2.0	37.8	46.9	5.8	5.8	6.4	Good
Baled	11.4	2.0	37.5	43.3	5.8	3.9	4.8	Fair

are important factors in the transformation of carbohydrates. Low content of water and high temperature tend to increase the polysaccharides and to decrease the monosaccharides; the reverse conditions tend to produce opposite trends (3). That the sugar levels in the 1945 samples did not follow this generally accepted pattern, seems added evidence that, as already suggested, unfavorable haying weather was a greater influence than adverse weather while the hay crop was growing.

Some evidence is available regarding the effect of various methods of curing on composition of the hay. Most of the samples had been field cured in the usual fashion but a few of them had been field baled and some had been cured by completing the drying with forced ventilation in the barn. Results of this phase of the study appear in table 3. All of the samples so cured are from the 1945 crop. They are compared in the table with similar kinds of hay field cured in ordinary fashion, and of the same year's crop.

The results here do not uniformly favor any method of curing; although the data are rather meager, it seems probable that factors other than the curing method *per se* were of more importance in affecting composition of the hays.

SUMMARY

In an attempt to determine the reason for lowered feeding value of much of the 1945 hay crop grown in this region, twenty-five samples of hay from five localities scattered over this state (Massachusetts) were analyzed. In addition to the usual proximate fodder analysis, total sugars and carotene were determined.

In comparison with samples of the 1943 and 1944 crop, hay samples from the 1945 crop were, in general, lower in sugar, carotene, ether extract, and total ash, and higher in fiber. Protein in most cases was average or above.

The results are discussed in the light of the weather records for 1945, an exceptionally wet season, and it is tentatively concluded that insufficient sunshine while the hay was growing, and unfavorable harvesting weather, were in large measure responsible for its relatively low content of such desirable entities as sugar, carotene, and minerals.

A rather close correlation was noted between quality of the hay as judged by appearance of the samples and farmers' opinions of its feeding value on the one hand, and its apparent desirability as revealed by chemical studies on the other hand.

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SHARK MEAL AS A PROTEIN SUPPLEMENT IN DAIRY CALF RATIONS[1]

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Shark meal is a relatively new livestock feedstuff which has been found to contain an average of 78.07 per cent of crude protein and which is high in calcium and phosphorus contents. The production of shark meal has been reviewed briefly by Marshall and Davis (9). During the past 6 years the output of this product in Florida and along the Pacific seaboard has reached significant proportions.

The value of fish meals as protein supplements in rations of young dairy calves has been reported by several investigators, but no literature has been observed on the evaluation of shark meal as a protein source for calves. Since sharks, class *Chondrichthyes*, whose skeletons are cartilaginous, are different from the bony fishes, class *Osteichthyes* (6), information was needed on the feeding value of shark meal as a protein supplement in rations for dairy calves.

LITERATURE

Little is known of the protein requirements of dairy calves under 6 months of age. Although many rations have been used satisfactorily to supplement milk during the first few weeks of life and later to replace it in the dietary of the young calf, few studies have yielded information on the minimum amount of protein required to produce an optimum rate of growth through 6 months of age.

Early feeding standards for dairy calves were formulated by Wolff, Lehman, and by Armsby. However, in discussing these standards, Eckles and Gullickson (3) pointed out that many of the data from which these recommendations were derived are of limited or doubtful value as applied to growing dairy calves. The minimum digestible crude protein allowance recommended for young dairy calves by the Morrison standard (11) increases from 0.24 pound daily at 100 pounds live weight to 0.61 pound at 250 pounds live weight.

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[1] **Taken from data presented in a thesis to graduate faculty of the University of Minnesota by Sidney P. Marshall in partial fulfillment of the requirements for the degree of Doctor of Philosophy.**

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At the Cornell station (5), Holstein calves fed *ad libitum* on a ration containing 8.3 per cent of crude protein (dry basis) produced very slow growth. Those fed rations containing 11.7 and 15.2 per cent of crude protein grew at a rate slightly below the Ragsdale normal (12), and an 18.8 per cent crude protein ration produced gains slightly above the normal. Nitrogen balances conducted on male calves approximately 10 and 15 weeks of age showed about equal nitrogen storage on the rations containing 15.2 and 18.8 per cent of crude protein, but an appreciably lower level of retention by those fed the other 2 protein levels. Harris (4) stated that the minimum requirement of Holstein calves 8 to 16 weeks of age lies between 11 and 14 per cent of crude protein in the ration.

For the investigation reported herein, the experimental rations were formulated to contain 14 per cent of crude protein, a level thought to be near the minimum which would support a normal rate of growth in dairy calves 8 to 24 weeks of age. Roller process powdered skim milk was selected as a protein source of recognized good quality with which to compare the feeding value of the nitrogenous constituents present in shark meal.

EXPERIMENTAL PROCEDURE

Several Jersey heifer calves at the Florida Agricultural Experiment Station were raised through 7 weeks of age on limited amounts of whole milk and a simple dry ration containing shark meal as the protein supplement. On the basis of age, weight, and general thrift, at about 8 weeks of age, 12 calves were paired and started on the experiment.

The basal rations were composed of prairie hay (No. 1 federal grade) ground to pass through a ¼-inch screen, 9 per cent of citrus molasses (1), 3 per cent of mineral mixture, and equal parts of ground yellow corn and ground oats. To the basal ration, the necessary amount of shark meal or of powdered skim milk was added to raise the total crude protein content of the ration to 14 per cent. Corn starch was included in the shark meal rations to adjust the quantity of nitrogen contributed by this protein supplement per pound of total feed to equal that per pound of total feed supplied by the powdered skim milk to the other rations. To comply with changes in the capacity of the young calf's stomach to utilize roughage, the proportion of hay to concentrates was altered with advancing age to approximate the records of hay and grain mixture consumption reported by Eckles (2) for Jersey calves raised by a limited milk feeding plan. The rations fed (table 1) contained 35 per cent of prairie hay during the first 4-week period, 40 per cent during the second, 45 per cent during the third, and 50 per cent during the fourth.

Rations containing shark meal, designated as rations 1, were fed to calves 1, 3, 5, 7, 9, and 11. Those containing powdered skim milk, designated as rations 2, were given to calves 2, 4, 6, 8, 10, and 12. The calves were paired as follows: 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12. Feed consumption of the calves of each pair was equalized by the paired feeding technique (10).

TABLE 1

Ingredients of calf rations used in comparing shark meal with powdered skim milk as a protein supplement

Ingredient	Rations 1 (%)	Rations 2 (%)
Period 1		
Ground prairie hay	35.0	35.0
Citrus molasses	9.0	9.0
Ground yellow corn	12.7	12.7
Ground oats	12.7	12.7
Mineral mixture*	3.0	3.0
Shark meal	14.63	
Corn starch	12.97	
Powdered skim milk		27.60
Period 2		
Ground prairie hay	40.0	40.0
Citrus molasses	9.0	9.0
Ground yellow corn	9.55	9.53
Ground oats	9.55	9.53
Mineral mixture*	3.0	3.0
Shark meal	15.30	
Corn starch	13.60	
Powdered skim milk		28.94
Period 3		
Ground prairie hay	45.0	45.0
Citrus molasses	9.0	9.0
Ground yellow corn	6.37	6.4
Ground oats	6.37	6.4
Mineral mixture*	3.0	3.0
Shark meal	13.33	
Corn starch	16.93	
Powdered skim milk		30.20
Period 4		
Ground prairie hay	50.0	50.0
Citrus molasses	9.0	9.0
Ground yellow corn	2.46	2.46
Ground oats	2.46	2.46
Mineral mixture*	3.0	3.0
Shark meal	14.12	
Corn starch	18.96	
Powdered skim milk		33.08

* The mineral mixture was composed of: defluorinated superphosphate, 48%; sodium chloride, 20%; potassium chloride, 13.3%; magnesium oxide, 13.3%; ferric chloride, 4%; manganous sulfate, 0.67%; copper sulfate, 0.28%; cobalt sulfate, 0.15%; zinc oxide, 0.15%, and potassium iodide, 0.15%.

Ten milliliters of shark-liver oil containing approximately 9,000 International Units of vitamin A per gram were mixed with a portion of the afternoon's rations on each Tuesday, Thursday, and Saturday.

TABLE 2

Biweekly live weights and total gains of calves

Week of experiment	Rations 1 (containing shark meal)							Rations 2 (containing powdered skim milk)						
	Calf 1	Calf 3	Calf 5	Calf 7	Calf 9	Calf 11	Average	Calf 2	Calf 4	Calf 6	Calf 8	Calf 10	Calf 12	Average
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Initial	86.5	95.0	78.5	94.5	92.5	92.5	89.9	90.0	93.5	86.5	98.0	78.0	92.5	89.8
2	92.0	119.0	97.5	100.5	101.5	104.0	102.4	92.0	108.0	97.5	103.0	85.0	106.0	98.6
4	111.0	124.0	108.5	107.5	106.0	107.0	110.7	110.0	116.0	110.0	106.5	92.0	108.5	107.2
6	121.0	130.0	120.0	116.0	124.5	128.0	123.3	121.0	119.0	107.5	119.0	113.0	132.0	118.6
8	129.0	148.5	133.0	142.0	133.5	149.5	139.3	132.0	141.0	131.0	143.0	127.0	148.5	137.1
10	146.0	172.0	152.5	160.0	155.5	162.5	158.1	145.0	157.0	146.5	164.0	146.5	168.5	154.6
12	159.0	185.0	171.5	177.5	175.0	180.0	174.7	154.0	182.0	163.5	182.5	161.5	181.5	170.8
14	189.0	207.0	183.0	188.0	185.0	188.5	190.1	183.0	191.0	179.0	195.0	175.0	199.0	187.0
16	208.0	229.5	205.5	209.0	195.0	203.0	208.3	204.0	222.5	202.0	214.0	194.0	205.0	206.9
Total gain	121.5	134.5	127.0	114.5	102.5	110.5	118.4	114.0	129.0	115.5	116.0	116.0	112.5	117.1

The calves were housed in individual pens in a barn on a concrete floor bedded with excelsior. Except for a few days of inclement weather, the animals were kept from noon until 4 o'clock each afternoon in an outside lot with concrete floor.

The calves were fed twice daily and water was kept before them at all times. Live weights were taken prior to the afternoon feeding the day before the experiment was begun and at weekly intervals thereafter. Weights taken on 3 successive days at the close of the experiment were used to calculate the final weight. On each weigh day, measurements were taken of the height at withers. For each animal, the average of 3 measurements falling within a range of 1 centimeter was used as the height.

RESULTS

The average initial live weight of the calves fed the shark meal rations was 89.9 pounds; those that received powdered skim milk rations averaged 89.8 pounds. Differences in initial body weight between individuals of each pair ranged from 0 to 14.5 pounds.

After 16 weeks of experimental feeding, the 6 calves on shark meal rations averaged 208.3 pounds in body weight and those fed powdered skim milk rations averaged 206.9 pounds. Differences in final body weights of pair mates ranged from 1.5 to 13.5 pounds. Live weights at biweekly intervals, average biweekly weights of all calves fed each ration, the total gain of each calf, and the average gain of the calves fed each ration are shown in table 2.

At the beginning of the experiment, the average height at withers was

TABLE 3

Weekly feed consumption for each calf and the average for calves fed each ration

Week of experiment	Calf 1 or 2	Calf 3 or 4	Calf 5 or 6	Calf 7 or 8	Calf 9 or 10	Calf 11 or 12	Rations 1 or 2
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	14.1	20.0	21.0	17.0	17.0	20.8	18.3
2	19.0	26.5	26.5	21.5	21.0	26.0	23.4
3	21.3	27.0	27.0	28.0	28.0	28.0	26.6
4	29.0	28.0	28.0	28.0	28.3	41.3	30.4
5	35.5	29.0	29.0	44.0	43.8	52.5	39.0
6	41.0	45.0	45.5	52.5	53.5	52.5	48.3
7	42.5	54.8	54.0	54.5	56.0	53.0	52.5
8	49.0	55.5	56.0	54.3	55.0	48.0	53.0
9	48.5	57.5	56.5	49.0	48.0	52.5	52.0
10	47.3	49.5	53.0	52.5	52 5	54.5	51.6
11	49.0	59.5	59.5	54.5	55.0	57.0	51.7
12	49.0	61.0	60.5	57.0	57.0	57.0	56.9
13	53.0	65.0	64.0	53.0	60.5	64.0	59.9
14	62.5	64.0	63.0	59.0	64.0	70.0	63.8
15	70.0	71.0	71.0	63.0	70.0	70.0	69.2
16	75.0	77.0	77.0	67.5	70.0	74.0	73.4

Note: When the weekly feed consumption of the calves in each pair was not equal, an average value is given.

72.4 centimeters for the animals placed on the shark meal rations and 72.8 centimeters for those on powdered skim milk rations. During the 16 weeks of the experiment, the average height of each group increased to 88.7 centimeters.

Feed consumption of the calves fed each ration averaged 18.3 pounds for the first week of the experiment. By the final week, the level of feed intake had increased to an average of 73.4 pounds. The weekly feed consumption of each calf and the average for the calves fed each ration are presented in table 3.

The composition of the calf rations as calculated from the analyses of their ingredients is presented in table 4. Rations fed during the same periods are similar in composition. During the successive periods, 1 through 4, there were increases in crude fiber and reductions in nitrogen-free extract in the rations. These changes in composition were caused by the periodic augmentations in the proportion of prairie hay in the rations.

TABLE 4

Composition of calf rations as calculated from analyses of ingredients

Ration number	Water (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Nitrogen-free extract (%)	Ash (%)	Ca (%)	P (%)	Mg (%)
Period 1									
1	14.88	14.00	2.61	10.96	48.28	9.50	1.439	0.540	0.458
2	12.70	14.00	2.03	11.00	51.26	9.00	1.102	0.547	0.448
Period 2									
1	14.78	14.00	2.44	12.01	47.32	9.69	1.481	0.541	0.440
2	12.49	14.00	1.83	12.06	50.45	9.17	1.130	0.548	0.429
Period 3									
1	14.19	14.00	1.74	13.05	48.68	8.99	1.283	0.509	0.390
2	12.29	14.00	1.62	13.12	49.61	9.34	1.156	0.548	0.411
Period 4									
1	14.06	14.00	1.48	14.04	47.98	9.13	1.318	0.503	0.448
2	13.00	14.00	2.74	14.06	46.39	9.81	1.221	0.621	0.434

DISCUSSION

The amount of feed offered was limited in each pair by the quantity that the calf with the poorer appetite would consume. Shark meal and powdered skim milk rations appeared to be equally palatable, and feed allowances were determined as frequently by the calves fed rations 1 as by those fed rations 2.

During the first few weeks on the dry experimental rations, feed consumption was low and calves gained slowly. Following an increase in feed consumption during the fourth week, the growth rate improved but continued slightly below the normal. At times during the ninth through eleventh weeks in the experiment, most of the calves had mild cases of diarrhea. Inappetence occurred in the affected calves, and the average feed consumption of the animals did not increase during this period. The average rate of gain and the weekly feed consumption of calves fed each of the rations is shown in figure 1.

The calves fed the shark meal rations made an average gain of 118.4 pounds in body weight and 16.3 centimeters in height at withers. The pair mates fed the powdered skim milk rations gained 117.1 pounds and increased 15.9 centimeters in height at withers. Neither the difference in mean gain between the two groups nor the difference in increase in height at withers is significant at the 5 per cent level (13).

Chemical analyses of the shark meal used during the periods 1 and 2 revealed that 1.264 per cent of nitrogen was present in the form of urea, and in a fraction of non-protein nitrogen other than urea. The shark meal used during periods 3 and 4 contained 1.421 per cent of nitrogen in these forms. These non-protein compounds contain a larger percentage of nitrogen than do proteins. Therefore, when the conventional factor N × 6.25 is used to convert the nitrogen of shark meal into crude protein, the value obtained exceeds the total amounts of nitrogenous constituents present. As a result, that total of the feedstuff analyses of the shark meal rations slightly exceeds 100 per cent (table 4).

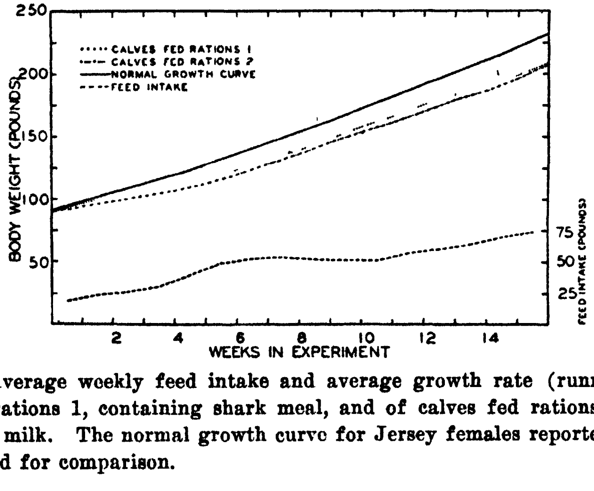

FIG. 1. Average weekly feed intake and average growth rate (running averages) of calves fed rations 1, containing shark meal, and of calves fed rations 2, containing powdered skim milk. The normal growth curve for Jersey females reported by Ragsdale (12) is included for comparison.

Researches by many investigators have indicated that ruminants, through the medium of rumen flora and fauna, are able to use some non-protein nitrogen in the dietary. Loosli, McCay, and Maynard (8) and Loosli and McCay (7) observed that Holstein calves approximately 2 months of age were able to utilize some urea as a source of dietary nitrogen. Calves weaned at approximately 2 months of age and continued on a basal ration containing about 4.4 per cent of protein gained an average of 7 pounds in 2 months, while those receiving 4 per cent of urea in the basal ration were 75 to 90 per cent of the normal in weight at 4 months of age. At 3 to 4 months of age, calves fed the low-protein diet were in a negative nitrogen balance, while those fed the basal diet plus urea were able to store nitrogen. These investigations indicate that the non-protein nitrogen present in shark meal may have been utilized by the calves in the studies reported herein.

SUMMARY

Shark meal was compared with roller process powdered skim milk as a protein supplement in simple rations fed to Jersey calves. Using the paired feeding technique, 6 pairs of females were fed 8 to 24 weeks of age on rations containing 14 per cent of protein.

The 6 calves fed the shark meal rations made an average gain of 118.4 pounds in body weight and 16.3 centimeters in height at withers. Their pair mates fed the powdered skim milk rations gained 117.1 pounds and increased 15.9 centimeters in height at withers. The difference in growth rate is not significant at the 5 per cent level. The average rate of growth was slightly below the normal reported for Jersey females.

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COLOSTRUM MILK AND ITS VITAMIN A CONTENT

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INTRODUCTION

In 1933 Dann (1) suggested that the calf received from twenty to fifty times as much vitamin A from colostrum as from an equal volume of whole milk. He observed that the vitamin A content of colostrum varied widely in cows of the same breed receiving identical rations and that the vitamin A potency of the colostrum from five heifers studied was double the average of that of nine older cows. Stewart and McCallum (2) related certain types of infections in young calves to the vitamin A content of the colostrum from their dams. These workers (3) observed a hundred cows from several herds, which presumably received similar rations, and suggested that the length of the dry period prior to parturition significantly effected colostral vitamin A. They reported no difference due to the age of the cow. Wide variations in vitamin A content of colostrum have been reported by Dann (1), Semp *et al.* (4), Stewart and McCallum (3) and others (5, 6, 7).

Vitamin A deficiency was observed in cows on the classical experiments of Hart *et al.* (8) and its effect on the newborn calf was noted. Jones *et al.* (9) produced a vitamin A deficiency in the calf and recorded the symptoms. Krauss (10) analyzed the livers of newborn calves and found them low in vitamin A. Phillips *et al.* (11) observed symptoms in young calves under certain farm conditions that responded to vitamin therapy, and further, as a result of blood plasma studies it was concluded that calves are born uniformly deficient in vitamin A. Sutton *et al.* (12) observed a decrease in blood plasma vitamin A in the cow concurrent with its appearance in the colostrum. It is generally assumed that colostrum supplies adequate amounts of vitamin A to meet the needs of the very young calf. The appearance of vitamin A deficiency symptoms in calves, including low blood plasma A concentrations suggests that a low vitamin A content of the colostrum may be responsible for these symptoms. We are aware that this suggestion does not preclude the possibility of poor absorption of the vitamin A present in the colostrum.

In an attempt to obtain further information on this subject a study was made of the vitamin A content of colostrum milk. Our approach to the problem was to determine the variation in vitamin A of colostral milks from cows which were maintained and milked under uniform conditions.

EXPERIMENTAL

Feeding of cows. Eighteen first-calf heifers were available for observation. Without access to pasture, they were fed and maintained throughout

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the experiment on a ration composed of medium quality alfalfa hay, corn silage, and a grain mixture (ground yellow corn 550, corn gluten 200, iodized salt 10 and bone meal 8). They were placed under observation at three to four weeks of age and were given rations, that were identical from the standpoint of vitamin A potency, in such proportions as to supply one-third of the daily requirement for T.D.N. respectively, from the grain, hay and silage. The heifers were bred to freshen at approximately thirty months and again at forty-two months of age, with a dry period of approximately two months between lactations. Other colostrum samples were available from Holstein, Jersey, and Guernsey cows from the regular university herd at different times throughout the year as well as from a few farm herds.

Collection of colostrum samples. Only those samples were used which were obtained from cows *milked out completely.* The cows were milked out at the first regular milking period following parturition and every twelve hours thereafter. Representative samples were taken of the first two milkings and if it was not convenient for immediate analysis they were frozen and stored in a darkened room at −4° C.

Method of analysis. It was found that little loss occurred in vitamin A potency if the milk was kept in the dark in a frozen state for periods up to thirty days. In these experiments analyses were made within fourteen days of collection. The milk was thawed with the aid of warm water and homogenized with a small hand homogenizer. Vitamin A and carotene determinations were made according to the method of Boyer *et al.* (13).

RESULTS

Heifers as compared to cows. Table 1 shows the individual carotene and vitamin A values of the colostrum obtained from eighteen Holstein cows that had been maintained under uniform conditions. The colostral vitamin A concentration of the milk of the first lactation was found to be more than double that of the second lactation. There was little difference in the average carotene values of the colostrum from the two lactations, despite individual variation.

Variation in cows of the same age. Table 1 also shows a wide variation in colostral vitamin A potency, between cows of the same age *that had been fed identical rations* for a two-year period previous to parturition. In the first lactation a range in vitamin A concentration of 70–480 micrograms per 100 ml. of colostrum was observed or about a seven-fold variation between cows fed and managed alike. Carotene potency showed much less variation. In general a high vitamin A potency colostrum was accompanied by a high carotene content.

Effect on the calf. Table 2 is a summary of data relative to the calves of the second lactation from the cows whose colostral A analysis were given in table 1. The calves were removed from the cows at birth. The cows were milked out completely at regular twelve-hour intervals and the milk

TABLE 1

The effect of lactation on the vitamin A content of colostrum

Cow No.	Vitamin A*		Carotene*		Days dry
	1st lactation	2nd lactation	1st lactation	2nd lactation	
1	410	58	132	59	40
2	199	176	148	230	61
3	70	A	75	A	
4	370	167	197	236	46
5	189	175	77	196	51
6	393	A	96	A	
7	480	234	200	212	60
8	355	179	228	195	57
9	321	A	177	A	
10	190	A	100	A	
11	330	56	143	79	54
12	475	135	176	119	52
13	292	X	99	X	
14	301	74	89	105	87
15	195	127	126	124	58
16	313	138	181	173	61
17	214	156	131	181	57
18	439	57	130	69	49
Ave.	308	133^{13}	139	152^{13}	

* Ave. first two milkings μg./100 ml.
A = aborted.
X = This cow was slaughtered before she freshened.

was sampled for vitamin A analysis then given to the calves at the rate of 12 pounds of milk per 100 pounds of body weight. Blood plasma vitamin A determinations were made on the calves before feeding, at one day of age, and on the 7th and 14th days respectively. On the 14th day the calves were weighed.

It is again clearly demonstrated by the data in table 2 that the calf is born with an extremely low but somewhat variable blood plasma vitamin A

TABLE 2

The effect of the level of vitamin A ingested on the blood plasma vitamin A level and growth of the calf

	Micrograms per 100 ml.				Pounds
	Initial blood blood plasma vitamin A	Colostral vitamin A intake	Increase in blood plasma vitamin A (24 hours)	Ave. blood plasma vitamin A values for period	Gain 2-week period
4	2	167	9	11	10
5	3	175	8	9	0
7	2	234	12	11	10
8	6	179	3	12	16
11	6	56	7	15	18
12	1	135	6	8	12
15	5	127	9	10	17
16	2	138	5	7	8
17	6	156	10	15	22
18	4	57	2	8	10

concentration. This is in agreement with previous observations and with those of the Maryland, and the Ohio workers (14, 7). After the ingestion of colostrum the blood plasma concentration of vitamin A increased markedly. There was an apparent difference in the ability of the calves to utilize the vitamin A present in the colostrum. However, in cases where the colostral A was high the blood plasma vitamin A usually reached a satisfactory concentration. There appeared to be a relationship between the average blood plasma vitamin A level during the 14-day period and the amount of weight gained by the calf in the same period.

TABLE 3

The effect of milking procedure on the vitamin A content of the colostrum

Lot	I	II	III
No. cases	13	4	8
	Vit. A. *μg./100 ml.*		
1st milking	131	239	318
2nd milking	135	134	226

Effect of milking procedure. Samples of colostrum were available from Holstein, Jersey and Guernsey cows that received three different types of treatment following parturition. All cows were milked out completely with the aid of a milking machine at twelve-hour intervals. The cows in lot I were isolated from their calves. The cows in lot II were allowed to remain with the calves throughout the experimental period. The cows in lot III were isolated from their calves but they were given 10 I.U. of oxytocin intravenously at the time of milking to stimulate complete letdown of milk. Colostrum samples were taken at the first, second, third, sixth, and tenth milking in all groups.

Table 3 shows the average colostral vitamin A values from the first and second milkings for these three groups. Although individual variation was noted, it appears that cows treated with oxytocin (lot III) and cows left with their calves in addition to being milked out (lot II) produced colostrum of a much lower vitamin A potency in the second milking as compared to the first. This was not the case with cows which were isolated from their calves and milked out in a normal manner, since the average vitamin A potency of the colostrum from the first and second milking of these cows was the same. It should be pointed out, however, that the initial vitamin A level in the colostrum from the cows in lot I was lower than that from the cows in lots II and III. This may be accounted for by feeding differences as these cows were maintaned under barn feeding conditions (without pasture) throughout the year while the cows in lots II and III were allowed access to pasture.

Change from colostrum to normal. Table 4 shows the rate of change from colostrum to normal milk with respect to vitamin A, and represents

TABLE 4

The rate of change in vitamin A concentration from colostrum to normal milk

	No. of milking					Normal
	1	2	3	6	10	
Carotene in I.U./10 lbs. milk	63,500	38,440	20,200	13,600	6,000	1,720
Vitamin A in I.U./10 lbs. milk	52,600	35,200	19,500	14,800	9,700	3,140

the average analyses of the twelve cows comprising lots II and III (see above). The control milk vitamin A concentration figures were obtained by analysis of milk from six of these cows in mid lactation. There was essentially no difference between lot II and III in the rate of change with reference to vitamin A levels in colostrum to that of normal milk. The data show that the main drop in vitamin A occurred during the first three milkings.

DISCUSSION

These results are in agreement with the work of Dann (1) and Henry *et al.* (5) who suggested that the colostrum from heifers was a richer source of vitamin A than the colostrum from older cows. If the length of the dry period affects the vitamin A potency of colostrum as reported by Stewart and McCallum (3), it is surprising that these workers did not observe a difference in colostral A potency between heifers and older cows.

It should be emphasized that cows of uniform age which had received identical rations varied widely in the vitamin A content of their respective colostral milks. Assuming that the intake of the calf during the first day was ten pounds of milk the variability in vitamin A intake by the calf would be from 10,000–86,400 I.U. per day. The lower range of vitamin A ingestion calculated here has been shown to be borderline in meeting the vitamin A requirements of the newborn calf (15). Add to this the hazards of limited nursing, congestion of the udder and nursing habits of the young calf and it is evident that some calves which receive colostrum may be deficient in vitamin A and may at times exhibit various deficiency symptoms.

SUMMARY

A study of the vitamin A concentration of colostral milk has been made. The vitamin A content of the colostrum from barn-fed heifers in the first lactation was found to be more than double the vitamin A content of the colostrum from the same cows in the second lactation. A seven-fold variation in colostral vitamin A potency occurred in these cows which had been fed identical rations and maintained under uniform conditions.

A marked increase in the blood plasma vitamin A concentration of the newborn calf followed the ingestion of colostrum and tended to reflect the concentrations of vitamin A present in the colostrum of their dams.

With cows isolated from their calves the vitamin A potency of their colostrum of the first and second milkings was about the same. However, cows which were allowed to remain with their calves and in addition were milked out twice daily showed a decided drop in vitamin A potency between the first and second milking. The intravenous injection of 10 I.U. of oxytocin at the time of milking in cows isolated from their calves also produced a similar drop in vitamin A potency between the first and the second milking.

The chief drop in concentration of vitamin A in the change from colostrum to normal milk occurred during the first three milkings following parturition, thereafter there was a gradual drop in vitamin A concentration over a period of several days to the concentration found in milk produced in mid-lactation.

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THE EFFECT OF CERTAIN COAL-TAR DYES USED FOR SEMEN IDENTIFICATION ON THE LIVABILITY AND FERTILITY OF BULL SPERMATOZOA*

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In the routine use of artificial breeding of dairy cattle it is impossible to distinguish the semen of one breed from that of another by any characteristic exhibited by the semen itself. Thus, in the handling of diluted semen of the various breeds, semen of one breed might inadvertently be used to inseminate cows of a different breed. Such mistakes are particularly serious in purebred herds where the registration of offspring is involved.

Recently Phillips (2) has reported data on the effect of certain vital dyes upon the motility of bull spermatozoa. He found that the addition of Nile blue sulfate, neutral red and Sudan III in sufficient amounts to distinctly color the diluent caused no marked harmful effects on spermatozoan motility over a storage period of several days. However, he stated that Sudan III was an inferior coloring agent due to its insolubility in water. Methylene blue, Janus green, thionin and alizarin sulphonate dyes reduced the motility of the spermatozoa to the extent that they could not be recommended for coloring purposes. No field data on the fertility of semen colored with vital dyes have been reported.

Since coal-tar dyes used for coloring foods for human consumption must be uniformly free from harmful impurities, studies were undertaken to determine whether certain of them could be used to color diluted semen. Laboratory and field studies were made to test their effects on the livability and fertility of bull spermatozoa.

EXPERIMENTAL

Preliminary studies indicated that coal-tar dyes certified for general use in foods, drugs, and cosmetics (FD&C colors) offered a simple means of coloring semen under field conditions. These dye preparations can be obtained commercially in liquid form.[1] Since semen diluted with an egg yolk-buffer is yellow in color, it was necessary to resort to the use of shades or tints. The following certified FD&C liquid colors were found to give adequate color differentiation when added to diluted semen; (1) Strawberry Shade Red Color (pure dye content not less than 5.2%), (2) Emerald Shade Green Color (pure dye content not less than 2.7%), (3) Purple Shade Grape

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[1] Supplied through the courtesy of the Warner-Jenkinson Manufacturing Company, 2526 Baldwin Street, St. Louis 6, Missouri.

Color (pure dye content not less than 4.1%), and (4) Brown Color (pure dye content not less than 3.7%). These liquid colors contain two or more of the certified dyes commonly known as Amaranth, Ponceau SX, Ponceau 3R, Tartrazine, Fast Green FCF and Brilliant Blue FCF. The brown color also contains a substantial amount of caramel (burnt sugar color).

The dry dyes used in preparing the colors mentioned above are manufactured according to Federal specifications covering identity and purity, and each batch is individually certified by the Federal Food and Drug Administration. The liquid colors used in these studies are water solutions of the various certified FD&C dry colors and contain as a preservative, to prevent mold growths, 1/10 of 1 per cent sodium benzoate and enough food grade phosphoric acid to lower the pH to approximately 3.7. These liquid colors are additionally certified after preparation, as required by Federal regulations concerning the manufacture of such colors made for use in food products.

The above certified liquid colors are standardized as to strength, are uniform as to color intensity and can be stored for reasonably long periods of time. Since aqueous solutions made from certified dry FD&C colors must be used soon after preparation, unless a suitable preservative has been added, they are not as satisfactory for practical purposes as certified liquid FD&C colors which store well and require no further preparation before use.

Livability during storage. To determine the influence of these coal-tar dyes on the livability of spermatozoa under laboratory conditions, 15 fresh semen samples diluted at the rate of one part of semen to 10 parts of an egg yolk-citrate diluter (4) were colored, using one drop of dye solution for each 10 ml. of diluter. The diluted semen was gradually cooled to the storage temperature, 40° F., and the percentages of actively motile spermatozoa were determined every 2 days for a period of 10 days.

The 15 individual ejaculates had a mean concentration of 1,099 thousand spermatozoa per cubic mm., and a mean methylene blue reduction time (1) of 7.6 minutes.

The results of the motility observations are shown in table 1. Each figure represents a mean of fifteen ejaculates. Analysis of variance (5) of the motility data (table 1) involving 375 separate motility observations showed no significant differences between dyes. Highly significant differences ($P = < 0.01$) between ejaculates and between storage intervals were found, as well as a highly significant storage interval × ejaculate interaction.

Field study of fertility. After the foregoing experiments upon spermatozoan livability were completed, a study was conducted to determine the effects of these color preparations upon the fertility of semen used under routine artificial breeding conditions. The Latin square experimental design was employed in order to avoid bias in the selection of semen samples to be used with any particular dye, and to eliminate in so far as possible

TABLE 1

The effect of certain coal-tar dyes on the livability of spermatozoa during storage

Treatment	Per cent motile spermatozoa (15 ejaculates)					
	Before storage	After storage at 40° F. for				
		2 days	4 days	6 days	8 days	10 days
Control	68	63	49	40	30	23
Red	68	61	48	39	29	21
Green	68	60	49	39	30	22
Brown	68	62	47	38	31	24
Purple	68	61	48	39	31	23

any inherent differences in fertility between the bulls, as well as any time trends in the fertility of the bulls (3). A 5 × 5 Latin square composed of 3 Holstein and 2 Guernsey bulls of the Nepa Artificial Breeding Cooperative, Tunkhannock, Pa.,[2] and five treatments, including the uncolored control and the red, green, brown, and purple color preparations was used. The first experiment (I), involving 5 one-week collection periods, was conducted from December 9, 1945, to January 12, 1946, and was immediately followed by the second (II) or replicate experiment which ran from January 13 to February 16, 1946. Thus the design called for 25 collections in each Latin square, or a total of 50 for the study. Therefore, 10 collections were used with each color treatment.

Whenever possible, only one semen ejaculate was collected from each bull during each weekly collection period. However, if the volume or quality of the first sample collected did not meet the requirements of the Cooperative for breeding purposes, a second ejaculate was collected and either used to replace the first ejaculate or mixed with it before dilution and the mixture considered as one sample in the experiment.

Following collection of the semen, data on the initial motility of the spermatozoa, the number of spermatozoa per cubic mm., and the methylene blue reduction time were obtained. Next the semen was diluted with egg

TABLE 2

Mean data on the quality and dilution rate of the semen used in the field study

	Treatment				
	Control	Red	Green	Brown	Purple
Sperm/mm.^{3} (thousands)	1,538	1,541	1,738	1,640	1,496
Motility (%)	78	76	77	77	76
Methylene blue reduction test (minutes)	4.4	4.2	4.4	4.5	4.7
Motility survival (days)	23	22	25	21	22
Ratio of semen to diluter	1: 17	1: 17	1: 18	1: 16	1: 17

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[2] The author wishes to express his appreciation to William F. Schaefer, Jr., Manager, for carrying out the details of the field trial.

TABLE 3

The effect of certain coal-tar dyes on the fertility of semen

Experiment	Treatment														
	Control			Red			Green			Brown			Purple		
	S	NR	%	S	NR	%	S	NR	%	S	NR	%	S	NR	%
I	316	229	72.5	286	196	68.5	269	197	73.2	281	206	73.3	281	196	69.8
II	344	238	69.2	311	216	69.5	289	214	74.0	292	208	71.2	326	218	66.9
Total	660	467	70.8	597	412	69.0	558	411	73.7	573	414	72.3	607	414	68.2

S = Total of 1st and 2nd services.
NR = Total 2 months' non-returns.
% = Percentage of 2 months' non-returns.

yolk-citrate prepared according to the design of the experiment, and gradually cooled to 40° F. The diluted semen was utilized by two technicians operating out of the headquarters of the central organization, as well as shipped to six affiliated local artificial breeding cooperatives. From 1.0 to 1.5 ml. of diluted semen were used per insemination and the semen was usually deposited in the uterus.

The mean data on the quality and dilution rate of the semen used in this study are shown in table 2. Each figure is a mean of 10 ejaculates. The average rate of dilution was 1 : 17, with a range of from 1 : 11 up to 1 : 24.

In the livability study a higher concentration of dye solution than needed for satisfactorily coloring the semen was used. In the field trial only enough of the four coal-tar dyes tested was used to give adequate color distinctions. Thus one drop of the red, green, and brown color preparations was added for each 25 ml. of egg yolk-citrate diluter or major fraction thereof (or 0.2 ml. of dye solution per 100 ml. of diluter) while one drop of the purple shade was added for each 15 ml. of diluter.

Table 3 presents mean data on the fertility of dyed and undyed semen used for insemination purposes. A total of 2,995 inseminations, of which 2,376 were first services and 619 were second services, were involved in this study. An average of 59.9 first and second service cows were inseminated with each diluted semen sample. The smallest number of cows inseminated with any one sample was 12, while the highest was 107. In only 4 cases out of the possible 50 were the number of cows inseminated less than 25.

Analysis of variance of the fertility data (table 3) based on the percentages of first and second service cows which did not return for service within 60 days showed no significant differences in the fertility of the colored and uncolored semen samples. The highly significant difference ($P = < 0.01$) found between bulls was expected since bulls of different fertility levels were selected for the study.

DISCUSSION

The experiments presented in this paper demonstrate that certain coal-tar dyes may be used as a practical means of coloring diluted bull semen. When the red, green, brown, and purple shade dye solutions were added to diluted semen there was no significant reduction in the ability of the spermatozoa to maintain their motility during storage.

Since a field trial would serve as a more conclusive means of testing the usefulness of these dyes, a study was designed using only enough of the several dye solutions to give adequate color differences. On the basis of 2,995 inseminations, or an average of 59.9 cows inseminated per sample of diluted semen, no significant differences between treatments were found.

Upon completion of the field trial the technicians were asked to give their reactions concerning the use of the particular dyes tested. Reports received agreed that the red and green dye solutions gave the best color differentiation, while purple was the next most desirable. Brown was the least popular color. Since the completion of this experiment, the Nepa Artificial Breeding Cooperative has been routinely coloring all diluted Holstein semen green and all Jersey semen red, while Guernsey semen remained undyed or yellow owing to the presence of egg yolk in the diluter used. Ayrshire semen will be colored purple when bulls of that breed are added to the stud.

No practical difficulty has been experienced with fading of the coal-tar colors when added to diluted semen. Recently a sample of green vegetable color was obtained in liquid form from a commercial source. When added to diluted semen, it was observed that even though a distinct green color was imparted to the semen initially, the color faded very rapidly, whereas the colors obtained with semen treated with coal-tar dyes remained very stable.

SUMMARY

Experiments were conducted to determine whether certified coal-tar dye solutions could be used to color diluted semen in order to reduce errors in its handling. Red, green, brown, and purple color preparations were tested under laboratory and field conditions. Spermatozoa in diluted semen colored at the rate of one drop of the basic red, green, brown, and purple dye solutions for each 10 ml. of diluter maintained their motility as well as those in the uncolored controls.

In a field study, when the same dyes were used at a lower concentration, no significant differences in fertility were found between the colored and uncolored semen samples. This investigation involved 2,995 inseminations and an average of 59.9 cows were inseminated per semen sample.

The results obtained demonstrate that the coal-tar color preparations described can be safely used as a practical means of coloring diluted bull semen.

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INFLUENCE OF NONFAT DRY MILK SOLIDS ON THE NUTRITIVE VALUE OF BREAD

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Wheat flour is a good food and an excellent source of energy, which is one of the first requirements that must be adequately met in a diet, and since white bread may contribute an appreciable proportion of the required calories to the American dietary, considerable effort has been expended toward improving its nutritive value. Modifications of standard milling processes so as to retain more of the nutritive value of whole wheat (particularly in Canada and the British Isles); the addition of nonfat dry milk solids; the use of yeast products having a high vitamin potency, and more recently enrichment, are all measures which have been suggested to improve the nutritive value of white bread. The use of nonfat dry milk solids has had wide acceptance because in addition to improving the nutritive qualities of white bread it also improves the physical properties.

Fairbanks (1, 2) first demonstrated the value of nonfat dry milk solids in improving the nutritive value of white water bread. His results have recently been confirmed by Mitchell, Hamilton, and Shields (9) and Light and Frey (8). Henry, Houston, Kon, Powell, Carter, and Halton (7) have attributed the better nutritive value of white bread made with nonfat dry milk solids to an increase in quantity and quality of protein and increased amounts of calcium, riboflavin, and possibly other members of the B_2 complex. Harris, Clark, and Lockhart (6) and Volz, Forbes, Nelson, and Loosli (11) found that milk solids and soybean flour and combinations of these two supplements improve the nutritive value of white bread protein.

The studies here reported were undertaken for the purpose of making comparisons of the nutritive values of several types of bread largely used in this country, viz.: water bread, enriched water bread and two whole wheat breads which differed considerably in composition on account of the flour from which each was made. Each of these types of bread was studied with and without the addition of 6 per cent nonfat dry milk solids. Mitchell *et al.* (9) studied white bread enriched according to the standards proposed in 1941 (Federal Register (3)). Since that time new enrichment standards have been promulgated (Federal Register (4, 5)) and it seemed important to conduct studies at the new enrichment levels.

METHODS

Young rats of the Sprague-Dawley strain weighing 35 to 50 grams were used in these studies. They were housed in individual cages and weighed

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twice weekly. Weighed portions of air-dried bread crumbs, mixed with water so as to prevent scattering, were fed daily. The bread crumbs, water, and a daily dose of 0.1 cc. USP cod-liver oil constituted the sole articles of diet throughout the 8-week test period. *Ad libitum* feeding was employed in Experiments No. 1 and No. 2. In Experiments No. 3 and No. 4 the animals were pair fed for equal gains.

Hemoglobin determinations were made on a Hellige clinical model hemoglobinometer using a sample of blood taken from the tip of the rat's tail. The values reported represent the average of duplicate determinations. A.O.A.C. (10) analytical methods for bread were used in determining the chemical composition of the diets and recognized chemical and microbiological methods were used in determining the amounts of iron and vitamins The carcasses were prepared and analyzed in the following manner: At the end of the eight-week growth test the animals were killed, the intestinal contents removed, and the entire carcasses were quickly frozen. The frozen carcasses were ground three times with a food chopper, being adequately mixed and refrozen after each grinding. Appropriate aliquots of the entire ground carcasses were taken for chemical analyses following the methods for meat given in the A.O.A.C. (10).

DESCRIPTION OF BREAD SAMPLES

Usually enough bread was baked at one time to last throughout each experiment. The breads were prepared using the following formula which is much like a commercial baker's formula. Sponge: 58 per cent flour, 2 per cent yeast, 0.5 per cent bread improver and 41.6 per cent water. Dough: 42 per cent flour, 4 per cent sugar, 2 per cent salt, 2 per cent malt, 1.5 per cent shortening (hydrogenated vegetable oil) and 27.7 per cent water. The same lot of white flour (0.42 per cent ash) or whole wheat flour was used to prepare all the breads fed in any one experiment; the supplements being added at the time the bread was made.

The following types of bread were studied.

1. *Water bread.* White flour without supplement.
2. *Enriched water bread.* White flour enriched to 10 per cent above the minimum levels for thiamine, riboflavin, niacin, and iron as specified in the new standards for enriched flour (Federal Register (4)). Analyses of the bread crumbs (table 1) show that these amounts of enrichment ingredients produced bread enriched well above the new minimum requirements of 1.1 mg. thiamine, 0.7 mg. riboflavin, 10 mg. niacin and 8 mg. of iron per pound of enriched bread (Fderal Register (5)).
3. *Nonfat milk bread.* 6 per cent nonfat dry milk solids based upon weight of white flour.
4. *Enriched nonfat milk bread.* A combination of the supplements given under 2 and 3.

5. *Whole wheat bread.* 100 per cent whole wheat flour.

6. *Whole wheat nonfat milk bread.* 6 per cent nonfat dry milk solids based upon weight of whole wheat flour.

Two different lots of whole wheat flour were used and the breads made from them showed differences in chemical composition as shown in table 1. For example, whole wheat bread #1 contained 17.13 per cent protein whereas whole wheat bread #2 contained 15.14 per cent protein.

Complete chemical and vitamin analyses were not made on all the bread samples fed. However, the same formula was followed wherever confirmatory tests were made and the analyses of each type of bread studied is shown in table 1. Every lot of bread was tested for moisture and protein. The analyses of the bread crumbs have been calculated to the basis of fresh bread containing 38 per cent moisture and these values are also shown in table 1.

RESULTS OF FEEDING EXPERIMENTS

The results from the feeding experiments are summarized in table 2. The relative nutritive values of these several types of bread can be fairly accurately evaluated on the basis of the grams of solids required to produce one gram of gain and this has been done as shown in the last column of table 2. When evaluated on this basis the smaller the weight of food required to produce a gram of gain, the higher the nutritive value of the food. The nutritive values calculated in this manner were subjected to statistical analysis in order to determine the significance of differences referred to in this report.

Experiments No. 1 and No. 2 (ad libitum feeding). The small differences between the nutritive values of water bread and enriched water bread are not significant when the data are analyzed by Student's "t" test for unpaired differences. (Experiment No. 1, $P > 0.9$; Experiment No. 2, $P = 0.4$).[1]

The addition of 6 per cent nonfat dry milk solids greatly improved the nutritive value of enriched water bread in both experiments, and the results from Experiment No. 2 indicate that water bread containing 6 per cent nonfat dry milk solids is equal in nutritive value to enriched water bread containing 6 per cent nonfat dry milk solids. Mitchell *et al.* (9) obtained similar results; however, they observed a slight but significant increase in hemoglobin levels when nonfat milk bread was enriched according to the 1941 standards. Hemoglobin determinations were not made on the animals used for this comparison in these studies.

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[1] The numerical value of P (Probability) indicates whether the difference between observed values are due to chance or to the imposed experimental conditions. When $P \leq 0.05$ (odds $\geq 19:1$) we consider the difference between two means to be statistically significant. When $P \leq 0.01$ (odds $\geq 99:1$) we consider the difference to be highly significant.

TABLE 1

Typical analyses of breads used in feeding trials

Identification	Moisture	Protein (N × 6.25)	Ash	Calcium	Phosphorus	Iron per lb.	Thiamine per lb.	Riboflavin per lb.	Niacin per lb.
Composition of dry bread crumbs									
	%	%	%	%	%	*mg.*	*mg.*	*mg.*	*mg.*
Enriched water bread	6.78	12.95	2.60	0.060	0.098	12.20	1.86	1.36	15.62
Enriched nonfat milk bread	7.15	14.25	2.87	0.120	0.139	12.00	1.80	1.86	15.57
Water bread	7.57	12.96	2.54	0.055	0.096	5.45	0.50	0.38	4.31
Nonfat milk bread	9.22	13.38	3.00	0.114	0.136	11.32	0.43	1.23	4.80
Whole wheat bread #1	10.45	17.13	3.39	0.065	0.234	22.60	1.36	1.27	18.06
Whole wheat nonfat milk bread #1	10.42	17.45	3.74	0.132	0.247	24.00	1.09	2.50	17.45
Whole wheat bread #2	10.75	15.14	3.34	0.070	0.415	17.60	1.75	0.80	17.80
Whole wheat nonfat milk bread #2	11.01	16.24	3.53	0.140	0.475	15.70	1.20	2.47	15.30
Composition of bread (38% moisture) calculated from crumb analyses									
	%	%	%	%	%	*mg.*	*mg.*	*mg.*	*mg.*
Enriched water bread	38.0	8.61	1.73	0.040	0.065	8.11	1.24	0.90	10.39
Enriched nonfat milk bread	38.0	9.52	1.92	0.080	0.093	8.01	1.20	1.24	10.40
Water bread	38.0	8.69	1.70	0.037	0.064	3.66	0.34	0.25	2.89
Nonfat milk bread	38.0	9.14	2.05	0.078	0.093	7.73	0.29	0.84	3.27
Whole wheat bread #1	38.0	11.86	2.35	0.045	0.162	15.64	0.94	0.88	12.50
Whole wheat nonfat milk bread #1	38.0	12.08	2.59	0.091	0.171	16.61	0.75	1.73	12.08
Whole wheat bread #2	38.0	10.52	2.32	0.049	0.288	12.22	1.22	0.56	12.36
Whole wheat nonfat milk bread #2	38.0	11.31	2.46	0.098	0.331	10.95	0.84	1.72	10.66

Nonfat milk bread was not equal in nutritive value to whole wheat bread #1. Also the addition of 6 per cent nonfat dry milk solids did not produce a significant improvement ($P = 0.12$) in the nutritive value of whole wheat bread #1. It should be noted that whole wheat bread #2 which was fed in Experiment No. 4 had a lower nutritive value.

TABLE 2

Summarized data from feeding tests

Diet	No. of rats	Av. initial weight	Av. wt. at end of 8 weeks	Av. gain	Av. wt. bread solids consumed	Bread solids required for one gram gain
Experiment No. 1 (*ad libitum* feeding)						
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
Enriched water bread	8	42.4	85.5	43.1	312.9	7.34
Water bread	8	43.0	90.6	47.6	340.7	7.25
Enriched nonfat milk bread	8	40.9	132.0	91.1	442.4	4.90
Experiment No. 2 (*ad libitum* feeding)						
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
Enriched water bread	8	41.0	79.2	38.4	296.8	7.85
Water bread	8	39.6	73.6	34.0	275.4	8.28
Enriched nonfat milk bread	8	40.5	100.5	60.2	367.6	6.15
Nonfat milk bread	8	39.0	98.0	59.0	355.6	6.10
Whole wheat bread #1	8	40.0	154.1	114.2	527.0	4.66
Whole wheat nonfat milk bread #1	8	41.5	192.6	151.1	638.8	4.30
Experiment No. 3 (paired feeding for equal gain)						
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
Enriched water bread	12	40.5	65.3	27.4	261.1	10.75
Water bread	12	40.4	63.8	22.6	269.1	12.10
Nonfat milk bread	12	38.3	80.7	42.3	279.8	6.61
Enriched water bread	12	38.8	76.5	37.7	333.9	8.86
Whole wheat nonfat milk bread #1	12	39.4	135.1	95.6	434.6	4.57
Whole wheat bread #1	12	39.0	130.6	91.6	486.6	5.35
Nonfat milk bread	12	38.3	101.4	63.2	380.3	6.13
Whole wheat bread #1	12	37.4	103.7	66.3	348.8	5.28
Experiment No. 4 (paired feeding for equal gain)						
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
Enriched nonfat milk bread	8	39.6	97.9	58.3	335.9	5.85
Whole wheat bread #2	8	40.5	100.6	60.1	334.3	5.62
Whole wheat bread #2	8	37.9	103.0	65.1	360.7	5.57
Whole wheat nonfat milk bread #2	8	38.5	104.8	66.3	332.1	5.02

Experiment No. 3 (paired feeding for equal gain). Since Experiment

No. 1 and Experiment No. 2 were conducted by the *ad libitum* feeding method, differences in the palatability of the several types of bread may have influenced the results. Also, some of the animals attained greater body weights than others and thus required a larger amount of energy for maintenance. Therefore, a number of these breads were fed in Experiment No. 3 wherein comparisons between two types of bread were made by feeding them to 12 pairs of rats for equal gains. As is indicated by the grouping of the summarized feeding test data from this experiment in table 2 the following comparisons were made:

1. Enriched water bread versus water bread.
2. Nonfat milk bread versus enriched water bread.
3. Whole wheat nonfat milk bread #1 versus whole wheat bread #1.
4. Nonfat milk bread versus whole wheat bread #1.

When compared by this method and the data analyzed by Student's "t" test for paired differencs, enriched water bread exhibited a nutritive value which was significantly higher than that of water bread ($P < 0.01$). Whereas we have been able to demonstrate a slight improvement in the nutritive value of water bread by enrichment to the new levels it should be noted that neither of these two types of bread has as high nutritive value as are exhibited by the other types of bread fed in these experiments.

The results on nonfat milk bread and enriched water bread are in agreement with those obtained in Experiments No. 1 and No. 2, and again show the superior nutritive value of water bread made with 6 per cent nonfat dry milk solids.

When fed for equal gains the addition of 6 per cent nonfat dry milk solids produced a significant improvement in the nutritive value of whole wheat bread #1 ($P < 0.01$). However, this lot of whole wheat bread still exhibited a higher nutritive value than nonfat milk bread ($P < 0.01$).

Experiment No. 4 (paired feeding for equal gain). It appeared that we had been using a whole wheat flour of unusually high nutritive value in Experiments No. 2 and No. 3. The whole wheat bread crumbs had a very high protein content (17.13%) and the results from the feeding tests did not agree with similar experiments reported in the literature (Mitchell *et al.* (9) and Light and Frey (8)). Therefore we repeated the experiments involving whole wheat bread using a whole wheat flour of lower protein content or one which more closely approached the average protein content of whole wheat flour. The breads made from this flour are referred to as whole wheat bread #2.

The results of this feeding test are also shown in table 2. The difference between the nutritive value of enriched nonfat milk bread and whole wheat bread #2 was not significant ($P > 0.1$) and the nutritive value of whole wheat bread #2 was improved by the addition of 6 per cent nonfat dry milk solids ($P < 0.01$).

CARCASS ANALYSES

Rate of growth on a diet and the nutritive value of the diet as measured by the grams of solids required to produce a gram of grain are generally recognized methods of evaluating a diet. However, further information about the nutritive value of a diet can be obtained from the chemical composition of the deposited tissue since this can vary in percentage of solids, fat, ash and protein. In order to evaluate some of the bread diets from this standpoint a number of the carcasses from experiments No. 3 and No. 4 were analyzed. Since the animals were pair fed for equal gain the analytical data were analyzed statistically by Student's "t" test for paired differences.

Experiment No. 3. The 12 pairs of rats which had been fed for equal gains on nonfat milk bread and enriched water bread were continued on the same dietary regime for two weeks following the 8 week growth test during which time hemoglobin determinations were made. The hemoglobin values, weights of the cleaned carcasses and results of the chemical analyses of the carcasses are summarized in table 3.

TABLE 3

Average hemoglobin values and carcass analyses—Experiment No. 3

Diet	Av. weight of cleaned carcass	Av. hemoglobin	Carcass analyses		
			Ash	Calcium	Nitrogen
	gm.	*gm./100 cc.*	%	%	%
Enriched water bread	88.7	13.27	2.79	0.68	3.070
Nonfat milk bread	92.2	12.96	3.35	0.88	3.172

The carcasses of the nonfat milk bread rats contained a significantly higher percentage of nitrogen ($P = 0.02$) and a significantly higher percentage of calcium ($P < 0.01$). The difference between the concentration of hemoglobin in the blood of the two groups of rats was not significant ($P = 0.3$). Fairbanks (2) showed that the addition of 6 per cent nonfat dry milk solids to white water bread increased the calcium content but not the nitrogen content of the carcass.

Experiment No. 4. The four groups of 8 rats each used in this paired feeding experiment were killed immediately following the 8-week growth test and the prepared carcasses were analyzed for total solids, fat, protein, and ash. The weights of the cleaned carcasses and their average chemical composition calculated to the dry basis are summarized in table 4.

The carcasses of the animals which had been fed enriched nonfat milk bread contained a significantly higher percentage of ash than the carcasses of the animals which had been fed whole wheat bread #2 ($P < 0.01$). Other

TABLE 4

Average carcass analyses—Experiment No. 4

Diet	Av. weight of cleaned carcass	Solids	Fat dry basis	Ash dry basis	Crude protein (N × 6.25) dry basis
	gm.	%	%	%	%
Enriched nonfat milk bread	94.0	34.10	32.94	9.79	56.96
Whole wheat bread #2	96.4	33.43	32.08	7.96	59.93
Whole wheat bread #2	99.5	33.78	33.72	7.65	57.96
Whole wheat nonfat milk bread #2	101.5	33.04	30.04	9.33	59.96

differences in the chemical composition of the carcasses of these two groups of experimental animals were not statistically significant. Therefore, it appears that enriched nonfat milk bread is equivalent in nutritive value to whole wheat bread of average composition both from the standpoint of weight gained per unit of bread solids consumed and from the chemical composition of the carcass produced. Judged on the basis of per cent ash in the carcasses, enriched nonfat milk bread seems to be nutritionally superior to whole wheat bread. Mitchell *et al.* (9) found that white bread made with 6 per cent nonfat milk solids was superior to whole wheat bread in the promotion of bone calcification.

The carcasses of the animals which had been fed whole wheat nonfat milk bread #2 contained a significantly higher percentage of ash than the carcasses of the animals which received whole wheat bread #2 ($P < 0.02$). Otherwise the differences in chemical composition between these two lots of carcasses were not statistically significant. These results confirm those of the growth experiment wherein whole wheat nonfat milk bread was found to have a higher nutritive value than whole wheat bread. It required less of the whole wheat nonfat milk bread to produce a one-gram gain, yet the carcass composition was equivalent to that of those fed the whole wheat bread in solids, fat and protein, and superior to those of the rats fed whole wheat bread in ash.

CONCLUSIONS

1. The addition of 6 per cent nonfat dry milk solids improves the nutritive value of water bread, enriched water bread and whole wheat bread.

2. Water bread supplemented with 6 per cent nonfat dry milk solids is superior in nutritive value to enriched water bread as measured by the grams of solids required to produce a gram of gain and the per cent nitrogen and calcium in the carcasses of the experimental animals. There was no significant difference between hemoglobin levels in the rats on which these comparisons were made.

3. Water bread supplemented with 6 per cent nonfat dry milk solids and enriched to the levels of the new standards is equivalent in nutritive

value to whole wheat bread of average composition as measured by grams of solids required to produce a one-gram gain and the chemical composition of the carcasses of the experimental animals.

4. Enrichment at the new levels causes a slight improvement in the nutritive value of water bread which can be observed by paired feeding for equal gain.

5. The improvement in the nutritive value of whole wheat bread brought about by the addition of 6 per cent nonfat dry milk solids has been confirmed by carcass analyses.

6. Whole wheat breads vary in nutritive value depending upon the type of whole wheat flour used.

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BLOOD STUDIES OF LOUISIANA DAIRY COWS. II. CALCIUM, INORGANIC PHOSPHORUS, HEMOGLOBIN VALUE, ERYTHROCYTE COUNT, LEUCOCYTE COUNT AND DIFFERENTIAL LEUCOCYTE PERCENTAGES

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Studies of animal blood have taken a prominent place in nutritional and physiological research. "Normal" blood values for dairy cattle have been published for calcium (1, 17, 25, 9, 22, 23), inorganic phosphorus (1, 15, 9, 12, 5, 24), hemoglobin (1, 13, 2, 14), and blood cells—erythrocyte count, leucocyte count and percentages of types of leucocytes (19, 4, 6). In general, there is a lack of agreement among the values reported for most of these constituents.

Many factors contribute to the differences in the average "normal" blood calcium and inorganic phosphorus values for dairy cattle. Among those factors can be listed types of methods and techniques such as using blood serum (1, 25, 5), blood plasma (15, 17, 9), or whole blood (12, 22, 24), dietary factors (16, 11, 22, 23), physiological factors (15, 24, 21, 22, 23), and many other factors, known and unknown (18, 15, 17, 10, 5).

Ferguson, Irwin, and Beach (6) found the variation in their study of the blood cells of healthy dairy cattle to be due particularly to differences between animals. They propose that "each animal of the species probably has a blood picture in health peculiar to itself, just as each has its own size, form, etc."

More accurate measurements are therefore necessary concerning the amount of variation for various blood constituents of dairy animals in different sections of the country.

This study was made to determine the variations in the blood picture over a period of time of high-producing dairy animals in two separate herds which were fed and managed better than the average. Also included is the blood picture of low-producing animals of an experimental herd on a basal diet which contained poor quality hay as the only source of roughage, supplemented with either calcium or phosphorus, or both calcium and phosphorus, or neither of these minerals.

EXPERIMENTAL

Determinations were made over a period of approximately two years, beginning in March 1944, on pooled citrated blood drawn on three consecutive days either monthly or bi-monthly from 10 Holstein and 6 Jersey

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cows of the L.S.U. herd at Baton Rouge, from 8 to 16 Jersey cows of the U.S.D.A. experimental herd at Jeanerette, and 14 Jersey cows of the experimental herd at L.S.U. Six of the original 16 animals in the L.S.U. herd, 12 of the original 16 animals in the Jeanerette herd, and all of the animals in the experimental herd were used continuously during the 2-year period. The remaining animals of the original 16 in the L.S.U. and Jeanerette herds were replaced by other animals in the herd during the 2-year period. Only 8 of the 12 original animals in the Jeanerette herd were continuously used for hemoglobin and cell counts. Analyses for calcium and phosphorus were made on 773 pooled samples of blood, while 613 pooled blood samples were used for determinations of hemoglobin, erythrocyte count, leucocyte count, and differential leucocyte percentages.

Calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method (3), phosphorus by the Fiske and Subbarow method (7) using a Coleman spectrophotometer, hemoglobin by the acid hematin method with a Klett-Summerson photoelectric colorimeter, standardized for 13.8 grams per 100 cc. of blood equal to 100 per cent. Total counts were made with standard hemocytometers and pipettes. Thick blood smears stained with Giemsa stain (8) were used for the differential counts of the various types of leucocytes.

A statistical analysis of the data was made according to Snedecor (20).

RESULTS AND DISCUSSION

Table 1 presents the plasma calcium, plasma inorganic phosphorus, and hemoglobin values for 46 dairy animals in three separate herds over a period of two years.

Calcium. The plasma calcium values for the animals in the L.S.U. herd ranged from 9.00 to 15.28 mg. per cent with a mean of 10.89 ± 0.21; for the

TABLE 1

The calcium, inorganic phosphorus, and hemoglobin values for Louisiana dairy cows over a 2-year period

Herd	No. of samples	Range	Mean S. E.
Calcium (mg. % plasma)			
L.S.U.	213	9.00–15.28	10.89 ± 0.21
Jeanerette	320	9.35–13.87	11.36 ± 0.15
Experimental	240	7.22–12.73	9.95
Phosphorus (mg. % plasma)			
L.S.U.	213	4.00– 8.80	5.77 ± 0.13
Jeanerette	320	3.50– 9.00	5.02 ± 0.07
Experimental	240	3.07– 9.44	6.14
Hemoglobin (gm./100 cc. whole blood)			
L.S.U.	213	8.99–18.60	11.59 ± 0.36
Jeanerette	160	8.91–15.86	11.19 ± 0.27
Experimental	240	9.60–18.25	13.39 ± 0.22

Jeanerette herd the range was 9.35 to 13.87 mg. per cent with a mean of 11.36 ± 0.15; and for the experimental herd the range was 7.22 to 12.73 mg. per cent with a mean of 9.95 mg. per cent. Since some of the animals in the experimental herd were receiving experimental rations supplemented with calcium and/or phosphorus, the standard errors for the mean plasma calcium and mean inorganic phosphorus values were not determined.

Much variation was present for the values for plasma calcium. Using analysis of variance, it was found for plasma calcium values that significant differences[2] existed *between animals* in a herd, and highly significant differences[3] existed *between months* (within animals). Nonsignificant differences were found between the mean calcium values for the three herds; and between the calcium values for Holsteins and Jerseys; and for the values between seasons of the year for each of the herds.

The mean plasma calcium values found are within the ranges reported by Robinson and Huffman (18), Palmer and Eckles (17), and Haag and Jones (9) who used blood plasma, although our calcium values for the various herds range slightly wider and higher. The calcium values for the animals in the experimental herd which were fed poor quality hay as the only source of roughage, with calcium supplement for some of the animals, are slightly lower than those of the better-than-average-fed herds, but not significantly lower.

The calcium values reported for blood serum by Anderson and co-workers (1) for calves and cows range from 9.96 to 16.18 mg. per cent, with an average of 12.63 mg. per cent. The average value for 5 cows was 13.31 mg. per cent. Figures reported for whole blood by Van Landingham *et al.* (22) range from 6.31 to 7.78 mg. per cent with an average of 7.06 mg. per cent.

Phosphorus. The plasma inorganic phosphorus values for the animals in the L.S.U. herds ranged from 4.00 to 8.80 mg. per cent with a mean of 5.77 ± 0.13; for the Jeanerette herd the range was 3.50 to 9.00 mg. per cent with a mean of 5.03 ± 0.07; and for the experimental herd the range was 3.07 to 9.44 mg. per cent with a mean of 6.14.

Significant differences[2] existed *between animals* in a herd and highly significant differences[3] existed *between months* (within animals) for the plasma inorganic phosphorus values. Nonsignificant differences were found between the L.S.U. and Jeanerette herds for the mean inorganic phosphorus values. For the experimental herd the mean inorganic phosphorus value was higher than and significantly different from those of the other herds. Nonsignificant seasonal variations were found for the inorganic phosphorus values for each of the herds, or between Holstein and Jersey values.

It is generally believed by dairy nutritionists that 4 to 6 mg. of inorganic phosphorus per 100 cc. of blood plasma are the normal levels for cows

[2] $P = 0.05$.

[3] $P = 0.01$.

(21). The inorganic phosphorus values for the L.S.U. and Jeanerette herds, 3.50 to 9.00 mg. per cent range somewhat higher than those reported in the literature for blood plasma by Robinson and Huffman (18), Palmer, Cunningham, and Eckles (15), and Haag and Jones (9), but the mean values are similar. Averages for inorganic phosphorus in blood serum as published by Anderson *et al.* (1) and Eveleth *et al.* (5) range from 4.46 to 8.02 mg. per cent, and in whole blood Johnson (12) and Van Landingham (24) report 4.3 mg. per cent for mature cows.

TABLE 2

The erythrocyte count, leucocyte count and differential leucocyte percentages for Louisiana dairy cows over a 2-year period

Herd	No. of samples	Range	Mean S. E.
Erythrocytes (million/cmm. whole blood)			
L.S.U.	213	2.57–9.94	5.27 ± 0.027
Jeanerette	160	3.00–7.48	4.89 ± 0.030
Experimental	240	3.03–9.60	5.72 ± 0.035
Leucocytes (per cmm. whole blood)			
L.S.U.	213	5,100–19,100	10,268 ± 45
Jeanerette	160	4,400–15,350	8,411 ± 31
Experimental	240	4,750–18,850	9,806 + 20
Differential leucocytes			
		Lymphocytes (%)	
L.S.U.	213	40–72	57.26 ± 0.79
Jeanerette	160	31–74	54.46 ± 0.86
Experimental	240	30–81	56.52 ± 0.74
		Neutrophiles (%)	
L.S.U.	213	10–58	29,35 ± 0.65
Jeanerette	160	16–52	32.64 ± 0.87
Experimental	240	11–68	30.23 ± 0.72
		Eosinophiles (%)	
L.S.U.	213	0–23	7.32 ± 0.37
Jeanerette	160	0–26	6.86 ± 0.37
Experimental	240	0–25	6.40 ± 0.38
		Monocytes (%)	
L.S.U.	213	0–17	5.49 ± 0.06
Jeanerette	160	0–16	5.67 ± 0.05
Experimental	240	0–19	6.38 ± 0.04
		Basophiles (%)	
L.S.U.	213	0–4	0.58 ± 0.08
Jeanerette	160	0–6	0.37 ± 0.09
Experimental	240	0–6	0.47 ± 0.06

Hemoglobin. The hemoglobin values for the three herds ranged from 8.91 to 18.60 mg. per cent, and the mean values ranged from 11.19 ± 0.27 to 13.39 ± 0.22 mg. per cent. Significant differences were found *between months* (within animals in a herd) for hemoglobin content, and also between the herds on an intra-month basis. Only low and insignificant correlations were found between either calcium or inorganic phosphorus and the hemoglobin values. Our values are within the range reported by other investigators (1, 13, 2, 14). Unless values are standardized on a percentage basis, they are difficult to compare since various methods use different standards.

Blood cells. Table 2 presents the number of erythrocytes and leucocytes, eosinophiles, monocytes, and basophiles for 38 dairy animals in three separate herds over a period of two years.

Significant differences *between animals* on an intra-herd basis were found only for the leucocyte count. Between-herd differences, however, were significant for erythrocyte count, leucocyte count, percentages of lymphocytes, neutrophiles and monocytes.

Only low and nonsignificant correlations were found between either calcium or inorganic phosphorus values and the erythrocyte or leucocyte counts.

Our erythrocyte counts are lower than values reported for purebred or grade Holstein cows and heifers by Ferguson, Irwin, and Beach (6) of Wisconsin who found a range of 4 to 10 million red blood cells per cubic millimeter with a mean of 6.33 ± 0.018 million, and 6.32 million red blood cells cited by Dukes (4). The mean leucocyte count for the animals of the L.S.U. herd and the experimental herd is higher than the value, 8,911 ± 42 reported by Ferguson *et al.* (6) and 7,900 cited by Dukes (4). No explanation as to why the erythrocyte counts are lower and the leucocyte counts higher in two of the herds can be given at this time. The mean percentages of lymphocytes, neutrophiles, and basophiles are between those cited by Ferguson *et al.* (6) and Dukes (4). The mean percentage of eosinophiles, 6.40 to 7.32 per cent, is slightly higher than the 5.0 per cent given by Dukes (4), but the 14.87 ± 0.13 per cent reported by Ferguson *et al.* (6) is twice as high as our figures, and almost three times as high as that cited by Dukes. The percentages of monocytes are slightly lower than the values reported (6, 4). According to Ferguson *et al.* (6), one explanation for the differences in percentages of types of leucocytes might be the staining technique used by the various investigators. They used the supravital staining method of Sabin (neutral red and Janus green); Dukes cited references that used Wright's stain; while in our study Giemsa stain was used.

Poor quality hay in the ration for the experimental herd did not seem to influence the amounts of the various cellular constituents.

Thus, differences in methods and technique employed, as well as physiological, environmental, and other factors are always present so that variations in "normal" values result. Isolated and single observations are sometimes used to reach certain conclusions, and are easily misinterpreted unless evaluated critically.

SUMMARY

Variations in the blood picture of high-producing dairy animals in two separate herds which were fed and managed better than average are pre-

sented. Also included is the blood picture of low-producing animals of an experimental herd which were on a basal diet containing poor quality hay as the only source of roughage, supplemented with either calcium or phosphorus, both calcium and phosphorus, or neither of these minerals.

Analyses were made on pooled citrated blood drawn on three consecutive days from 38 to 46 animals either monthly or bi-monthly over a period of approximately two years.

A statistical analysis of 773 determinations for calcium and phosphorus and 613 observations for hemoglobin, erythrocyte count, leucocyte count, and differential leucocyte percentages was made. Using the analysis of variance it was found that significant differences existed *between animals* in a herd and *between months* (within animals) for calcium and phosphorus values. There were significant differences *between months* (within animals) in a herd for hemoglobin values, and *between animals* for the leucocyte count. No significant seasonal variations were found. Only low and nonsignificant correlations were found between either calcium or phosphorus and the hemoglobin value, erythrocyte, or leucocyte counts. Significant differences were found between herds for hemoglobin content, erythrocyte count, leucocyte count, percentages of lymphocytes, neutrophiles and monocytes.

Poor quality hay in the ration of the experimental herd did not seem to influence the blood calcium content or amounts of the various cellular constituents, but the phosphorus and hemoglobin values were significantly different from those of the other herds.

The range of means and their standard errors for the herds were as follows:

Calcium	10.89 ± 0.21	to	11.36 ± 0.15	mg. per cent plasma
Phosphorus	5.02 ± 0.07	to	5.77 ± 0.13	mg. per cent plasma
Hemoglobin	11.19 ± 0.27	to	13.39 ± 0.22	gm. per 100 cc. blood
Erythrocytes	4.89 ± 0.030	to	5.72 ± 0.035	millions per cmm. blood
Leucocytes	8,411 ± 31	to	10,268 ± 45	per cmm. blood
Lymphocytes	54.46 ± 0.86	to	57.26 ± 0.79	per cent
Neutrophiles	29.35 ± 0.65	to	32.64 ± 0.87	per cent
Eosinophiles	6.40 ± 0.38	to	7.32 ± 0.37	per cent
Monocytes	5.49 ± 0.06	to	6.38 ± 0.04	per cent
Basophiles	0.37 ± 0.09	to	0.58 ± 0.08	per cent

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THE EFFECT OF COTTONSEED IN THE RATION ON PERCENTAGE OF FAT AND SERUM SOLIDS CONTENT OF MILK

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A number of studies have been made of the effect of different fats in the ration on the fat content of the milk (1, 2, 3, 4, 5, 8, 9, 10). Some of these were impractical because refined oil was used and in some instances fed in large amounts. Also, some experiments were not of sufficient duration to be conclusive.

Tests have been made with cottonseed meal and cottonseed oil but no reference is made to whole cottonseed. McCandlish (6) substituted cottonseed meal for both bran and linseed oil meal in rations of six cows—one Jersey, one Guernsey, and four Holsteins for 9 days of a 33-day period which alternated with 4 to 5 days in each of four check periods, 3 days in each of three experimental periods, and 2 to 3 days in the three transition periods, and noted very small changes in fat content and milk yield. This investigator also substituted cottonseed meal for the cracked corn in the ration with the result that a marked increase in the fat content occurred. Allen (1) supplemented rations with linseed oil, lard, corn oil, cottonseed oil, tallow, and butterfat in two 6-day periods using two Jersey, two Guernsey, and two Holstein cows, noted increases in butterfat production equivalent to 10 to 20 per cent of the additional fat fed. Allen and Fitch (2) noted marked increases when 1.5 pounds of butterfat, lard, or tallow were fed to two cows daily for 50 days. Cottonseed oil, linseed oil, cocoanut oil, or peanut oil produced small increases and soybean oil and corn oil produced decreased butterfat. Gibson and Huffman (3) do not concur with Allen's results when soybean oil was fed to two groups of four cows each in 24-day reversal periods. These investigators found that an increase in fat content of the milk was temporary, and the fat percentage returned to the normal level. Miller and Wise (7) found that at the end of a 4-month period, the milk produced by five to ten cows on a cottonseed meal ration had a lower fat content than the control. Woodward (10) conducted a series of 10-day tests and noted an increase in fat yield from 4.38 per cent during the control to 4.84 per cent when the four cows were fed cottonseed meal in amounts of 6.8 pounds to 11.2 pounds.

EXPERIMENTAL

Twelve cows—two Guernseys, four Jerseys, and six Holsteins—were divided into two groups with consideration given to breed, age, weight, period of lactation, and yield. Each group contained one Guernsey, two Jerseys, and three Holsteins. The experiment was conducted for three

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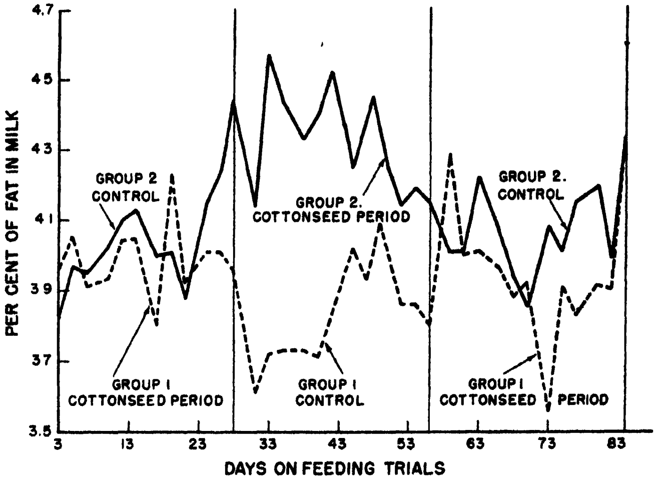

FIGURE I. PER CENT OF FAT IN MILK FOR EACH GROUP FOR THE TWO PERIODS.

FIG. 1. Percentage of fat in milk for each group for the two periods.

reversal periods of 28 days each, during February, March, April, and May. Group I received cottonseed during the first and third periods and Group II during the second period. The cows were machine milked twice daily.

The cows received all the alfalfa hay which they would eat. The cows all had pasture a few hours daily when available. The pasture consisted of barley, oats, rye grass, and alfalfa. A concentrate mixture of 630 pounds of rolled barley, 600 pounds of wheat bran, and 150 pounds of cottonseed meal was used. This was fed to the Jerseys at the rate of 1 pound for each 3 pounds of milk produced, Guernseys, 1 for each 4 pounds, and Holsteins, 1 for each 5 pounds of milk produced. This mixture contained 13.6 per cent digestible protein, 5.6 per cent fat, and 73.7 per cent total digestible nutrients. The only difference in the ration of the two groups was the replace-

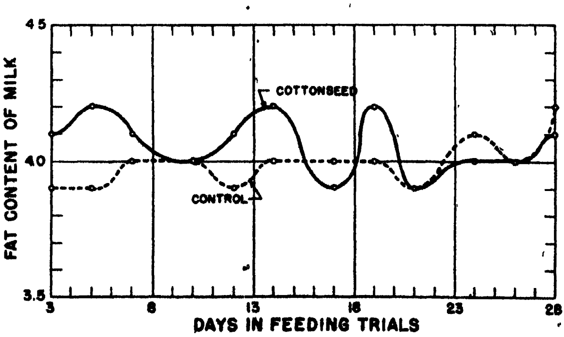

FIG. 2. Combined daily average fat percentage for the two groups for both the cottonseed and control periods.

ment of 2 pounds of the concentrate with 2 pounds of cottonseed. Cottonseed is reported, on 64 analyses (8), to contain 17.0 per cent digestible protein, 23.0 per cent fat, and 91.0 per cent total digestible nutrients.

When 2 pounds of the basal grain ration was replaced with a similar amount of cottonseed, the digestible protein of the mixture was increased 1.33 per cent and 0.91 per cent when fed in amounts from 5.2 to 12.2 pounds per cow, as shown in table 2. The fat content of the mixture containing cottonseed varied from 12.42 to 8.44 per cent in the above feedings. Total digestible nutrients were increased 6.78 and 2.83 per cent, respectively, by the replacement two of the 5.2 pounds of grain for Cow No. 141 and the 12.2 pounds for Cow No. 241.

Daily composite milk samples were taken Mondays, Wednesdays, and Fridays throughout the experiment and tested for fat—Babcock method, and total solids—Mojonnier method. The serum solids were determined by difference.

DISCUSSION

The data show that the replacement of 2 pounds of the concentrates with 2 pounds of cottonseed in the ration resulted in a temporary increase of fat in the milk. The production of Guernsey cow No. 34 is not included in the data because this cow refused to eat the cottonseed during the latter part of the experiment.

Table 1 shows the milk produced during the 28-day periods for each cow for both the cottonseed and control periods and the average per cent of fat, total solids, and serum solids.

TABLE 1

Milk produced and per cent fat, total solids and serum solids of the milk of each cow for the two periods

Cow No.	Cottonseed				Control			
	Milk	Fat	T.S.	S.S.	Milk	Fat	T.S.	S.S.
				Group I				
	lbs.	%	%	%	*lbs.*	%	%	%
122	765.3	5.16	14.09	8.93	778.0	4.78	13.82	9.04
141	429.4	5.70	14.86	9.16	439.2	5.70	14.86	9.16
219	1376.8	3.25	11.59	8.34	1469.8	3.12	11.54	8.42
225	1161.6	3.68	12.16	8.48	1202.4	3.63	12.13	8.50
237	1253.0	3.67	12.25	8.58	1267.7	3.61	12.15	8.54
				Group II				
	lbs.	%	%	%	*lbs.*	%	%	%
39	724.1	4.92	13.95	9.03	711.5	4.65	13.70	9.05
129	845.1	5.27	14.19	8.92	856.4	4.94	13.91	8.97
143	557.9	6.01	15.54	9.53	555.6	5.37	14.98	9.61
221	1079.3	3.81	12.23	8.42	1126.9	3.63	12.04	8.41
226	983.7	3.46	11.81	8.35	902.4	3.34	11.72	8.38
241	1363.5	3.59	12.12	8.53	1297.3	3.45	11.93	8.48

TABLE 2

Showing average daily milk production, amount of grain fed, and analyses of modified grain mixture when two pounds of cottonseed were substituted

Cow No.	Breed	Cottonseed period					Control	
		Average pounds milk per day	Pounds of modified grain ration	Per cent digestible protein	Per cent fat	Per cent T.D.N.	Average pounds milk per day	Pounds of basal grain ration
122	Jersey	27.33	9.1	14.34	9.42	77.50	27.79	9.3
141	Jersey	15.34	5.1	14.93	12.42	80.48	15.69	5.2
219	Holstein	49.17	9.8	14.29	9.15	77.23	52.49	10.5
225	Holstein	41.49	8.3	14.41	9.79	77.86	42.94	8.6
237	Holstein	44.75	9.0	14.35	9.46	77.54	45.28	9.1
39	Guernsey	25.86	6.5	14.64	10.95	80.25	25.41	6.4
129	Jersey	30.18	10.0	14.28	9.08	77.16	30.59	10.2
143	Jersey	19.92	6.6	14.63	10.87	78.94	19.84	6.6
221	Holstein	38.55	7.7	14.48	10.12	78.17	32.23	6.4
226	Holstein	35.13	7.0	14.57	10.57	78.64	32.23	6.4
241	Holstein	48.70	12.2	14.16	8.44	76.53	46.33	11.6

Table 2 shows the average daily milk production and the amount and analyses of the concentrates which each cow received. The digestible protein content of the modified grain ration was increased from 13.6 per cent to 14.16 to 14.93 per cent, the fat content of 5.6 per cent increased to 8.44 to 12.44 per cent, and total digestible nutrients increased from 73.7 per cent in the basal ration to 76.53 to 80.48 per cent.

The average fat test was higher during the cottonseed period for every cow except one. Jersey cow No. 141 tested the same for both periods. Jersey cow No. 143 gave the largest increase, viz., 0.64 per cent. The average increase of Group I was 0.13 per cent and that of Group II was 0.24 per cent.

There was not any material difference in the milk yield for the two periods. The total solids was slightly higher for the cottonseed period. This was caused by the higher fat. It is interesting to note that the cotton-

TABLE 3

Ratio of fat to serum solids

Group I			Group II		
Cow No.	Ratio fat : S.S.		Cow No.	Ratio fat : S.S.	
	Cottonseed 1 :	Control 1 :		Cottonseed 1 :	Control 1 :
122	1.731	1.891	39	1.835	1.946
141	1.607	1.666	129	1.692	1.815
219	2.566	2.698	143	1.585	1.789
225	2.304	2.341	221	2.209	2.317
237	2.337	2.365	226	2.410	2.509
			241	2.376	2.458

seed produced an increase in fat without a corresponding increase in serum solids. Table 3 gives the ratio of fat to serum solids for each cow for the two periods.

Table 4 gives the average daily milk production and average fat test for each cow for both periods. The fat tests for Group I averaged higher during the two cottonseed periods than the control period with variations during the 28-day periods of 3.80 to 4.23 per cent and 3.55 to 4.34 per cent as compared to 3.61 to 4.09 per cent during the control period. Group II likewise had a higher fat test during the cottonseed feeding period with tests of 4.14 to 4.57 per cent as compared to 3.83 to 4.44 per cent and 3.85 to 4.33 per cent in the two control periods.

TABLE 4

Average daily milk production and average fat test for each cow

Group I

	Cow 122		Cow 141		Cow 219		Cow 225		Cow 237	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
	lbs.	%	*lbs.*	%	*lbs.*	%	*lbs.*	%	*lbs.*	%
Cottonseed	27.33	5.16	15.34	5.70	49.17	3.25	41.49	3.68	44.75	3.67
Control	27.79	4.78	15.69	5.70	52.49	3.12	42.94	3.63	45.28	3.61
	−0.46	+0.38	−0.35	0.0	−3.32	+0.13	−1.45	+0.05	+0.53	+0.06

Group II

	Cow 39		Cow 129		Cow 143		Cow 221		Cow 226		Cow 241	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
	lbs.	%	*lbs.*	%	*lbs.*	%	*lbs.*	%	*lbs.*	%	*lbs.*	%
Cottonseed	25.86	4.92	30.18	5.27	19.92	6.01	38.55	3.81	35.13	3.46	48.70	3.59
Control	25.41	4.65	30.59	4.94	19.84	5.37	40.25	3.63	32.23	3.34	46.33	3.45
	+0.45	+0.27	−0.41	+0.33	+0.08	+0.64	−1.70	+0.18	+2.90	+0.12	+2.37	+0.14

There was a great fluctuation in fat tests during the cottonseed period. The test for this period was the same as that of the control on the tenth day, lower on the seventeenth day, and the same again on the twentieth. The test for the cottonseed period averaged slightly less than that of the control during the last 8 days of the experiment. The highest combined test for the cottonseed period was on the nineteenth day.

The results of this study are similar to those obtained by McCandlish (6) in which cottonseed meal increased the fat content of the milk for short periods. These findings are not in accord with those of Allen (1) who found no indication of a return to the normal level after six weeks of a high fat intake.

SUMMARY AND CONCLUSIONS

An experiment to study the effect of feeding cottonseed on the fat and serum solids content of the milk was conducted using twelve cows for three 28-day reversal periods.

1. The replacement of 2 pounds of the concentrates in the ration with 2 pounds of cottonseed produced an increase in the fat content for the first 20 days.

2. The fat test for the last 8 days of the experiment was slightly lower than that of the control. The fat test of the cottonseed feeding periods were higher, with a combined range of 3.55 to 4.57 per cent as compared to 3.61 to 4.44 per cent for the combined control periods.

3. There was no significant difference in the milk production for the two periods.

4. The cottonseed did not produce a corresponding increase in serum solids. In fact, the serum solids content was slightly reduced.

5. Palatability of cottonseed is a limiting factor in its use when mixed with rolled barley, wheat bran, and cottonseed meal.

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BABCOCK VS. MOJONNIER TEST FOR HOMOGENIZED AND UNHOMOGENIZED MILK

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The homogenization of a considerable portion of the market milk supply has become part of the processing procedure in many milk plants throughout the country. The value of homogenization has been given considerable attention in the literature. According to Hollingsworth (1), dairymen soon found that the Babcock test of pasteurized milk showed discrepancies with fat tests of the same milk after homogenization. The reason given for this difference was due to the decreased size of the fat globules in the homogenized milk. The minute globules could not be completely raised into the fat column by the action of sulfuric acid and centrifugal force in the Babcock test.

REVIEW OF LITERATURE

Trout (5) reported on 12 groups of Babcock tests on milk homogenized at 500, 1500, and 2500 pounds pressure at 90° and 145° F. The results show no practical or significant difference from unhomogenized milk. The milk homogenized at 2500 pounds resulted in fat tests 0.034 and 0.021 of a per cent lower for the respective homogenization temperatures. Hood and White (2) reported a variation of 0.08 of a per cent lower when the milk was homogenized at 3500 pounds pressure. Lower pressures gave less variation. Comparisons were made on 50 tests by Phillips (4) who reported that on unhomogenized milk, the Babcock test averaged 0.0588 of a per cent higher than the Mojonnier. No reference was made in the literature to comparisons of the Babcock and Mojonnier methods of determining butterfat content of homogenized milk.

EXPERIMENTAL PROCEDURE

Fat tests were conducted on unhomogenized milk and milk homogenized at 150° F. at 1500, 2000, 2500, 3000, and 4000 pounds pressure. The fat content of the milk varied from 3.2 to 4.4 per cent. The milk sample tested in duplicate by the Babcock method was pipetted directly into the milk test bottle. Sulfuric acid (sp. gr. 1.82) was added in three portions and shook twenty seconds aften each addition of acid. The mixture was also shaken after the first addition of hot water when centrifuging. No unsatisfactory tests resulted. The samples were tempered at 135° to 140° F. for five minutes before reading.

The Mojonnier tests were conducted in accordance with instructions

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TABLE 1

Showing comparative fat tests and the range of variations of unhomogenized and homogenized milk by the Babcock and Mojonnier methods

Pressure	Number of tests	Babcock				Mojonnier				Average difference between Babcock & Mojonnier	Extreme variations of the two methods
		Range of fat tests				Range of fat tests					
		High	Low	Aver-age	Vari-ation	High	Low	Aver-age	Vari-ation		
										%	
0	27	3.937	3.906	3.921	0.031	3.980	3.967	3.975	0.013	+0.054	3.906–3.980
1500	8	3.913	3.869	3.891	0.044	3.979	3.968	3.973	0.011	+0.082	3.869–3.979
2000	24	3.856	3.833	3.843	0.023	3.957	3.940	3.948	0.017	+0.105	3.833–3.957
2500	23	3.854	3.830	3.842	0.024	3.946	3.932	3.939	0.014	+0.097	3.830–3.946
3000	24	3.860	3.813	3.837	0.037	3.945	3.935	3.940	0.010	+0.103	3.813–3.945
4000	5	4.051	4.020	4.036	0.030	4.163	4.147	4.155	0.016	+0.119	4.020–4.163
Homogenized milk	84	3.873	3.841	3.857	0.032	3.966	3.952	3.959	0.014	+0.102	3.830–4.163

outlined by the manufacturer (3). The samples were pipetted into the extraction flasks.

RESULTS

Table 1 shows the results of the fat tests of unhomogenized milk and milk homogenized at the various pressures. The range of fat tests shows the variations between duplicate tests. The average of the high or original tests by the Babcock method for unhomogenized milk was 3.937 per cent as compared to the low or duplicate tests which averaged 3.906 per cent. The variation of the 27 pairs of samples was 0.031 per cent as compared to 0.032 per cent for 84 samples of homogenized milk tested in duplicate. The corresponding variations among unhomogenized and homogenized samples of milk by the Mojonnier method were 0.013 and 0.014 per cent, respectively.

The above-mentioned table also shows the difference in tests of unhomogenized and homogenized milks by the Babcock and Mojonnier methods.

DISCUSSION

The average test of 27 samples of unhomogenized milk was 3.921 per cent by the Babcock method as compared to 3.975 per cent by the Mojonnier method. This difference, in which the unhomogenized milk tested 0.054 of a per cent lower by the Babcock, is not in agreement with work of Phillips (4). This investigator reported the Babcock test of unhomogenized milk averaged 0.0588 of a per cent higher than the Mojonnier comparisons. The difference in fat tests between the Babcock and Mojonnier methods of milk homogenized at 1500, 2000, 2500, 3000, and 4000 pounds pressure were 0.082, 0.105, 0.097, 0.103, and 0.119 of a per cent, respectively. The difference between tests by the Babcock method of unhomogenized and homogenized milk is in agreement with results of Hood and White (2) who report that, at 3500 pounds pressure, the homogenized milk tested 0.08 of a per cent lower than the unhomogenized milk. Lower pressures resulted in correspondingly lower variations.

The variations between samples of milk tested by the Babcock method were essentially the same for both the unhomogenized and homogenized samples, the difference being 0.031 and 0.032 of a per cent, respectively. Table 1 also shows that the Babcock test gave lower results on homogenized milk. The milk homogenized at 1500 pounds pressure tested 0.030 of a per cent lower than the unhomogenized milk. Pressures of 2000, 2500, and 3000 pounds resulted in decreases of 0.078, 0.079, and 0.084 of a per cent for the respective pressures than unhomogenized milk.

Less discrepancies were noted in the range of fat tests by the Mojonnier method which show variations of 0.013 and 0.014 of a per cent for unhomogenized and homogenized milk, respectively. The homogenized milk also had fat tests with less deviation from the unhomogenized samples than the Babcock method.

SUMMARY

The results of 8, 24, 23, 24, and 5 samples of milk homogenized at 1500, 2500, 3000, and 4000 pounds pressure, respectively, were averaged. The extreme variation in fat tests by the two methods was noted in the samples homogenized at 4000 pounds, in which the Mojonnier results were 0.119 of a per cent higher than the Babcock. Samples homogenized at 1500, 2000, 2500, and 3000 pounds pressure had variations of 0.082, 0.105, 0.097 and 0.103 of a per cent, respectively. The average discrepancy between the two tests of the 84 samples of homogenized milk was 0.102 of a per cent. The Mojonnier method was higher in 80 of 84 samples of homogenized milk tested. The Babcock test was only slightly higher on four samples with average differences of 0.001, 0.005, 0.009, and 0.050 of a per cent.

The 27 samples of unhomogenized milk tested show that the Mojonnier method was 0.054 of a per cent higher than the Babcock. In 22 of the 27 samples, the Mojonnier tests averaged 0.058 of a per cent higher, and in the 5 remaining tests the Babcock exceeded by 0.038 of a per cent.

The Babcock test of the milk used in this study was 3.2 per cent to 4.4 per cent fat. No significant difference was noted in variations between these two methods of the 3.2 and 4.4 per cent milks.

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STORAGE AND TREATMENT OF MILKING MACHINE INFLATIONS*

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The storage and treatment of milking machine inflations has been given considerable attention and yet no single procedure for caring for them has been generally adopted. Chlorine and lye solutions have undoubtedly been most prevalently used where solution racks are employed, while dry storage following washing has been used by many producers.

Early investigation on the handling of inflations and rubber parts include treatment with steam and hot water and storage in solutions of vinegar, acetic acid, potassium permanganate, hydrogen peroxide, alcohol, "Gold Dust," formalin, salt, lime, borax, and chloride of lime. An excellent review of treatment as applied to rubber up until the year 1920 has been presented by Hart and Stabler (2). Of these early used sanitizing agents hot water and chlorine solutions have survived, while salt has also been used in a limited degree as an aid to prevent freezing when blended with chlorine or lye solutions. Steam, although effective as a germicide, was found by Stocking and Mason (7) to seriously injure rubber.

Deteriorating action by chlorine on rubber was noted by Hart and Stabler (2), who also showed that both milk and rubber reduced the chlorine content of solutions containing it. These workers advocated the use of hot water at 180° F. for 15 minutes as the most practical means of sterilizing under ordinary ranch conditions. Henderson, Roadhouse, and Folger (3) reported that heating rubber parts in hot water at 185° F. for 20 minutes was much more effective as a sterilizer than at 170° F. for the same period, and did not appreciably shorten the life of the rubber; though both heat treatments reduced the life of rubber about one-third when compared with chlorine. These workers failed to report deteriorating action on rubber from the use of chlorine although a solution strength of 200 p.p.m. of chlorine was used.

Lye at 0.3 to 0.5 per cent concentration was found by Parfitt (6) and Johns (4) effective in reducing bacteria counts on milk when used for storage solutions. More recent development in treatment of rubber inflations has included boiling them in lye solutions periodically, using four heaping teaspoonfuls of household lye for each quart of water. This procedure as developed by Moir (5) saponifies the rubber-entrained fat, thus restoring elasticity to the rubber.

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Observation made of rubber deterioration on milking machines on which data on washing procedure and results were being gathered suggested that further study of the various storage procedure should be made for the purpose of determining, (a) the effect of various chlorine and other storage solutions on synthetic rubber, (b) the fat absorption qualities of inflations and (c), other evidence calling attention to better care of milker inflations and rubber parts.

EXPERIMENTAL

Effect of sanitizing solutions on synthetic inflations. To determine the effect of sanitizing solutions frequently recommended for rubber inflation storage on synthetic rubber deterioration, twenty new inflations were branded with a number for identification; following which they were washed, dried for a period of 4 hours at room temperature and were then weighed. Sanitizing solutions were prepared and adjusted to correct concentration of parts per million in all the chlorine solutions used, as well as "Roccal" solution. The acid and alkaline solutions were prepared by weighing the amount required to produce a 0.5 per cent concentration. All solutions were prepared fresh. Once each week day one designated inflation from each jar was removed and worked by repeated stretching in fresh whole milk. This working of inflations in milk was regulated to one minute duration, following which they were thoroughly rinsed with clear tap water. At the close of each week all the inflations were removed, rinsed in clear water and again dried for a 4-hour period at room temperature, after which all inflations were weighed. Similar treatment was continued for thirteen weeks for all but two solutions, "Calgonite" and "Perfection," which were stored 65 and 42 days respectively.

The results are shown in table 1. Appreciable gain in weight occurred with storage in all solutions except lye. Greatest gain, however, occurred with the more highly active chlorine solutions, particularly "Lobax," "B K" and "H.T.H. 15." The "Nu Kleen" acid solution also resulted in gains practically as high as obtained with the more highly active chlorine solutions. Very slight gain in weight occurred in the lye solution. The significance of gain in weight was not ascertained. However, it is likely that excessive weight gains are associated with reduction in strength of rubber, for in extreme cases of swelling, such as was observed in connection with organic solvents, later reported in this study, the rubber was swollen and softened to the extent that it would tear with very little effort.

Significantly higher increases in gains of weight occurred in the milk-treated inflations than in those only treated in solutions. Also, deterioration as marked by softening and peeling of rubber occurred on milk-treated rubber when stored in "B K," "Lobax" and "H.T.H." solutions. Roughening of the rubber inflations as determined by scraping the inner surfaces with a metal-bladed spatula resulted with all chlorine storage when the milk treat-

TABLE 1

Showing effect of storing rubber inflations in different solutions on weight increase and rubber deterioration

Product	Solution strength	Number days held	Per cent wt. increase		Rubber appearance		
			Milk treated	Not milk treated	Color	Texture	Deterioration
I Sterichlor	200 p.p.m.	91		3.00	Shiny black	Smooth	
	200 p.p.m.	91	3.00		Shiny black	Sl. rough	
III B.K.	200 p.p.m.	91		2.59	Brown	Sl. rough	+
	200 p.p.m.	91	5.19		Brown	Rough	++
V L.Bax	200 p.p.m.	91		3.90	Brown	Sl. rough	
	200 p.p.m.	91	8.00		Brown	Rough	++
VII Diversol	200 p.p.m.	91		2.63	Black	Smooth	
	200 p.p.m.	91	4.40		Black	Sl. rough	
XIII H.T.H. 15	200 p.p.m.	91		2.77	Dull black	Smooth	
	200 p.p.m.	91	5.44		Dull black	Sl. rough	+
XI Nu Kleen acid	200 p.p.m.	91		3.98	Shiny black	Smooth	
	200 p.p.m.	91	5.02		Shiny black	Sl. tacky	
IX Roccal	200 p.p.m.	91		2.90	Shiny black	Sl. tacky	
	200 p.p.m.	91	3.15		Shiny black	Sl. tacky	
XV Lye	0.5%	91		0.29	Shiny black	Smooth	
	0.5%	91	0.55		Shiny black	Smooth	
XVII Calgonite	0.5%	65		2.53	Shiny black	Smooth	
	0.5%	65	2.17		Shiny black	Smooth	
XIX Perfection	0.5%	42		1.84	Shiny black	Smooth	
	0.5%	42	2.23		Shiny black	Smooth	

ment was applied. The "Nu Kleen" acid and "Roccal" solutions produced a tacky feel that was most noticeable when pressing the inner liners together and suddenly releasing the pressure. Color changes were noted. The color changed from an original shiny black to a brownish color as a result of the use of "B K" and "Lobax," while a dull black resulted from "H.T.H. 15." No apparent color changes occurred from the other solutions used. The slower chlorine-releasing products such as "Sterichlor" and "Diversol," would appear to be better suited for rubber storage than those of more active nature. The release of chlorine in an overly active solution can be materially inhibited by addition of tri-sodium phosphate as shown by Fay (1), who recommends that 100 grams trisodium phosphate be added to 1 gallon filtered calcium hypochlorite prepared from one 12-ounce can of chloride of lime.

Fat extraction from rubber inflations. The presence of fat in inflations has been previously shown by Moir (5), who demonstrated that inflations could be renovated by boiling in a strong lye solution.

The grayish appearance of the top half, or mouth end, of used milker inflations when stored for a period after discarding for reasons of breakage gave rise to the suspicion that fat absorption was greatest in that portion of the inflation that grips the teat. This, if so, would coincide with Moir's (5) reasoning that during the milking process a film of milk gathers between the teats and rubber, and some of the fat from the milk is gradually worked into the rubber.

As a means of determining the amount of fat that was worked into rubber, boiling with lye solution was tried, but no consistent results were secured when measured on a weight basis. This method was, therefore, discarded and organic fat solvents were decided upon. The solvents thought usable were ethyl ether, petroleum ether and Skelly Solve "A."

Extraction determinations were made on new synthetic rubber and latex rubber inflations to determine the amount of rubber that normally would be dissolved by the solvents during various time periods. These periods were selected as 2, 5, and 10 minutes when 100 ml. of each solvent was shaken with 10 grams of finely diced rubber particles. Another time trial was made for each kind of rubber using two 2-minute shaking periods, each with a 50-ml. portion of solvent. Following extraction, the extracts were poured into tared Mojonnier dishes and the solvents were evaporated in a Mojonnier vacuum oven. The weights of residue are shown in table 2.

From the results obtained it was apparent that synthetic rubber was more readily soluble than latex rubber in organic solvents. Also, it was seen that the amount dissolved was directly related to the time of exposure of rubber to solvent.

Of the solvents used petroleum ether was slightly lower in rubber dissolving action than Skelly Solve "A" and considerably lower in this respect

TABLE 2

*Showing grams of rubber dissolved from 10 grams of new milker inflation rubber when treated with various fat solvents**

Minutes shaken	Solvents					
	Ethyl ether		Petroleum ether		Skelly Solve "F"	
	Synthetic	Latex	Synthetic	Latex	Synthetic	Latex
2	0.2011	0.0718	0.1290	0.0577	0.1483	0,0485
5	0.2944	0.0967	0.1886	0.0748	0.2047	0.0729
10	0.4060	0.1603	0.2639	0.0938	0.2892	0.0941
2 + 2	0.2869	0.1037	0.1816	0.0657	0.1888	0.0682

* Results are average obtained on two series of trials.

than ethyl ether. Petroleum ether was, therefore, selected as the solvent to be used for making fat extraction determinations. Also, a period of five minutes was arbitrarily selected, as there was little difference in the duplicate determinations for any of the time periods used.

Fat extraction from used synthetic rubber inflation. To determine the amount of fat that could be extracted from used synthetic inflations the inflations were sectioned in such a manner as largely to remove the swollen and grayish black portions that showed signs of fat absorption. These were finely diced with razor blades and ten-gram portions were weighed and extractions made therefrom. The lower ends of the inflations were likewise diced and tested. Similarly inflations were tested that had been boiled in lye solution for 15- and 90-minute periods. This was done to determine the extent of fat removal by means of the lye treatment. The solution was made by adding 2 ounces of lye per gallon of water.

As a means of determining the amount of fat removed in these extractions the tare value of 0.1886 was applied to each. This being the amount dissolved on inflation rubber that had never been used. The results of these extractions are presented in table 3.

TABLE 3

Showing per cent of net fat extracted from upper and lower portions of used milker inflations

Inflation number*	Minutes boiled in lye sol.	Net percentage fat extracted from 10 grams of synthetic rubber milker inflations	
		Upper or mouth half	Lower half
		per cent	*per cent*
1	0	1.38	0.22
2	0	1.63	0.27
3	15	0.99	0.20
4	90	0.82	0.00

* Two determinations were made from each inflation section and the figures shown are their average.

Results from table 3 show that the upper portion of the inflation definitely absorbs fat to a greater extent than does the lower portion, although this lower portion is generally presumed to be more in contact with milk as it passes from the teat to the milker pail. Obviously the flexing of the rubber and the condition described by Moir (5), wherein a film of milk gathers between the teats and rubber, exists; thus resulting in milk fat being worked into the rubber.

Although no attempt was made in this trial to make tests for fat extraction of lye-boiled and nonboiled rubber from the same inflation, it was apparent that lye-boiling for 15 and 90 minutes reduced the fat content materially. However, despite boiling for a period of ninety minutes, the apparent amount remaining in the upper portion of the rubber was 0.82 per cent.

Fat extraction made from used latex rubber inflations. Extractions were made from sectioned latex rubber inflations similarly to synthetic inflations except that in order to make better determination of the influence of lye boiling, the inflations were divided longitudinally. Each half of these inflation parts was boiled in lye solution at 15; 30; and 90-minute intervals while the other halves were left unboiled. All halves were then transversely sectioned so that the distended portions indicating wear and fat absorption were separated from that of normal appearance. The line of demarkation generally appeared at approximately the midway portion of each inflation. To obtain the net fat extraction the tare figure of 0.0748 was deducted from all extraction weights. This being the amount of latex rubber extracted with petroleum ether in a 5-minute extraction period. Results are shown in table 4.

The results as presented in table 4 again show a decrease in fat as a result of boiling in lye solution and a greater fat reduction as the boiling treatment was increased. When boiling was applied for a period of 15 minutes 26.3 per cent of the total fat was saponified, with 30 minutes 40.7

TABLE 4

Showing per cent of net fat extracted from upper and lower portions of sectioned used latex rubber milker inflations when boiled and not boiled in lye solutions

Inflation number	Minutes boiled in lye sol.	Net percentage fat extracted from 10 grams of latex rubber milker inflations	
		Upper or mouth half	Lower half
		per cent	*per cent*
1	0	3.91	0.62
	15	2.88	0.31
2	0	5.79	0.72
	30	3.43	0.64
3	0	2.78	0.93
	90	1.11	0.37

per cent and with 90 minutes 60.1 per cent as determined from the losses of fat from the upper portions of rubber.

The total fat absorption was considerably higher in used latex rubber than in used synthetic. However, this may have been the result of longer period of usage in favor of the latex inflations. No record was obtained of the period of use of any of the rubber. Consequently, it could not be assumed that latex rubber was more fat absorbing than synthetic rubber.

Lye-boiling effect on heat-resisting bacteria. Since it was demonstrated that fat in substantial percentage was absorbed in inflations, it could be assumed that bacteria likewise could be absorbed with the fat and thus would impregnate the rubber in a manner that no amount of washing or surface treatment by chemical agents would be sufficient for adequate sanitization.

In substantiation of such a conjecture an actual instance is presented in which a short-tube-type milking machine was used. This milker was generally washed with only medium warm water and a high quality washing powder followed by rinsing with warm water to which a quarternary ammonia sanitizing agent was usually, but not always, added. On physical examination the milker pails and inflations appeared clean and entirely free of milkstone. Bacterial counts made bi-monthly for a period of several winter months on both raw and pasteurized samples of milk from this producer resulted in counts ranging from 127 thousand to 620 thousand in the raw samples and 85 thousand to 191 thousand in these samples after being laboratory pasteurized.

In order to determine the source of the bacteria that were not destroyed by pasteurization, arrangement was made with the owner to visit the farm during milking so that rinse counts could be made of the milking machine inflations before milking was begun and samples of milk taken for bacteria counts from each individual cow directly from the milker pails.

On the day of the tests the milking machines were washed in the house, whereas generally the washing was done in the milk house. The pail and the inflations had the appearance of thorough cleanliness.

The rinse samples were taken by removing the inflations from the milker pail head and pinching each inflation tubing to hold it shut so sterile rinse water could be held in the inflation until filled. The water was then allowed to run back into the jar. A 500-ml. portion of rinsing water was similarly applied to all milker inflations of a unit.

Despite the appearance of cleanliness noted in the milking machines the bacteria count of one set of inflations was over 10 million, while the laboratory pasteurized water sample had a count of over 7 million, both calculated as total count. In the second unit these counts were 4.5 million and 3.9 million, respectively. Also, all milk samples were high in both raw and pasteurized counts, with practically as high bacteria count in the milk from the last cows milked as in the milk from those milked first.

A week later a second series of tests were made. To assure having absolutely clean milking machines they were brought to the laboratory for cleaning. The milker pails were washed and rinsed with moderately hot water from a hose after which they were drained to dry. The rubber inflations were placed in a boiling lye solution, containing two ounces of lye per gallon of water, for a period of 30 minutes.

As a precaution udder samples were taken from the fore milk. These samples were taken aseptically by washing teats and udders with clean cloths dampened with chlorine solution. After discarding the first few streams of milk from each teat, milk was drawn directly into sterile test tubes. The samples were properly refrigerated and were plated within two hours. The results of bacteria counts made on the milker inflations and the milk from each cow as obtained before and after the lye boiling treatment are shown in tables 5 and 6.

TABLE 5

Bacteria counts secured per 500 ml. of rinsing water applied to four inflations of each milker unit

Inflations	Unit #1		Unit #2	
	Total	Thermoduric	Total	Thermoduric
Before boiling	10,500,000	7,500,000	4,500,000	3,900,000
After boiling	1,000	500	1,000	500
After rinsing following milking	60,000	1,000	25,000	500

Results in table 5 show the bactericidal effect on total and heat resisting bacteria when highly contaminated milker inflations were boiled in lye solution. The resulting bacteria counts of 1000 total and 500 thermoduric were considered excellent. While the bacteria counts were greatly improved the rubber also showed improvement in freshness and in general texture.

Rinse counts made after milking was completed and after the milking machines were washed with only moderately warm water containing a wetting agent washing powder are also shown in table 5. An increase in count over that secured with lye boiling showed such washing to be inadequate for satisfactory germ destruction. Sanitizing following washing with hot water at 180 to 185° F. or with satisfactory chemical solutions was unquestionably required.

Table 6 shows the beneficial effect obtained from boiling milker inflations in lye solution in reducing both raw milk and pasteurized milk bacteria counts. Further, these results established fairly definitely that the high thermoduric counts difficulty from this milking machine came from the inflations rather than other suspected sources, as the milker pails were in apparent excellent state of cleanliness when the first check-up was made and were not washed in the laboratory with more than normal care.

A direct effect of utensil contamination on bacteria count is shown by the

TABLE 6

Bacteria counts in milk from individual cows in the herd, showing counts secured before and after boiling apparently clean used inflations in a strong lye solution

Sample number	Bacteria count of milk					
	Before lye-boiling		After lye-boiling		Hand-milked fore milk	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
1	300,000	440,000	1,100	200	4,000	100
2	620,000	510,000	5,100	300	6,500	200
3	240,000	130,000	1,100	100	100	100
4	490,000	460,000	2,000	100	600	100
5	47,000	15,000	7,200	2,000	24,000	200
6	240,000	180,000	1,400	200	1,400	100
7	230,000	150,000	1,200	200	600	100
8	300,000	250,000	3,300	100	4,200	100
9	250,000	180,000	1,500	200	800	200
10	140,000	120,000	1,500	100	6,800	200
11	150,000	140,000	1,800	500	800	100
12	100,000	90,000	900	500	300	100
13	330,000	140,000	1,500	100	1,100	200
14	310,000	170,000	3,400	500	700	300

high counts in inflations and in milk in the first week's trials, while low bacteria counts in inflations and milk were obtained after the inflations had been boiled.

Contamination lodged in the inner pores of the rubber inflations would seem to be evident because bacteria counts were practically as high at the end of milking as at the beginning. Surface contamination only should result in a washing action with reduced counts as milking progresses.

TABLE 7

Bacteria counts secured from inflation rinses and subsequent milk supply

Date	Bacteria count of			
	Rinse water		Milk obtained on following day	
	Total	Thermoduric	Total	Thermoduric
1-29	3,600,000	15,000	160,000	97,000
2- 4	5,400,000	1,500	180,000	130,000
2-18	15,000,000	170,000	180,000	180,000
3- 5	1,000	500	180,000	180,000
3-19	3,000	1,000	270,000	200,000
3-26	30,000,000	10,000	130,000	100,000
4- 2	9,000,000	550,000	43,000	42,000
4- 4[*]	1,000	500	.	. . .
4- 9	73,000	1,500	40,000	10,000
4-15	3,000	1,500	40,000	1,500
4-23	3,000	500	120,000	7,000
4-30	2,000	500	15,000	5,500
5- 7	1,500	500	18,000	3,100
5-13	500	500	18,000	2,100
5-21	500	1,000	26,000	8,300

[*] Lye-boiled.

Following this washing experience the bacteria counts obtained on this producer's milk, both raw and pasteurized, and inflation rinses also "raw" and pasteurized, continued low and the pasteurized count relationship to the raw count was fairly normal. Bacteria counts as taken from Jan. 30, 1946, to May 8, 1946, are listed in table 7.

Special attention is called to the relationship existing between rinse counts before and after lye boiling in table 7. Generally the rinse counts made before lye boiling were extremely high. However, bacteria counts made on the milker inflations the days previous to taking milk samples on dates March 5 and 19 were at satisfactorily low levels with 1000 and 3000 recorded, respectively, for the untreated samples and 500 and 1000 for these samples after laboratory pasteurization. Despite these low bacteria counts made on rinses from inflations, the thermoduric bacteria counts were practically equal to counts from the raw milk. This was unlike the result obtained following boiling of inflations in lye solution. Although no samples from cans were taken the day following the lye-boiling treatment, tests made for each succeeding week continued low in the laboratory-pasteurized samples. It was, therefore, thought probable that the two low bacteria counts, secured previous to the boiling treatment, were not a true indication of the condition of the rubber inflations and that the surfaces of the inflations only were relatively free from bacteria. Also, it was thought probable that the pores of the rubber were infected and manifested this by contaminating the milk while flexing the rubber during the milking operation.

Unless a considerable amount of the contamination came from the pores, as well as from the surface of the rubber, it would seem that the milking process would produce a washing effect and would gradually cause a reduction in bacteria count. Since little if any actual progressive reduction took place it was concluded that much of the contamination during the period of high bacteria count was caused by lack of germicidal action in these pores.

Inasmuch as rubber is shown capable of absorbing various quantities of solutions in which it is stored it would appear likely that were sanitizing agents absorbed that would not be detrimental to rubber and would retain germicidal activity in presence of rubber and organic matter, such solutions would be useful for sanitary storage of rubber parts.

Such storage solutions should not be applied in place of proper cleaning, but rather as precaution against contamination from bacteria that are lodged in pores inaccessible to removal by washing treatment.

Since boiling inflations in lye solution proved beneficial in reducing bacterial contamination and removing butterfat from the rubber, such treatment at frequent intervals for all rubber that contacts milk is indorsed. It would appear that bi-monthly or monthly lye boiling treatment is sufficient when accompanied by thorough washing of the milker after each use.

SUMMARY

1. Absorption and deterioration of synthetic rubber milker inflations were studied.
    - (a) Synthetic inflations stored in sanitizing solutions were found to absorb various quantities of such solutions during the storage.
    - (b) Significantly higher quantities of solutions were absorbed when the inflations were treated by daily working in whole milk for a one-minute period followed by solution storage.
    - (c) Inorganic chlorine solutions caused greatest deterioration to synthetic inflations as exhibited by color change, roughening and dissolving conditions on the surface.
    - (d) Lowest absorption occurred when lye solution was used for storage.
    - (e) A slight tacky feel developed on the surface of inflations stored in acid and the cationic surface-active sanitizing agent, "Roccal."
2. A method for determining absorbed fat is described.
    - (a) Highest quantities of fat were absorbed in the mouth or upper portion of inflations.
    - (b) The amount of absorbed fat decreased as the lye-boiling period was extended.
3. Effective reduction was shown in number of heat-enduring bacteria by boiling inflations in a strong lye solution.

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A RAPID DIRECT-DISTILLATION METHOD FOR DETERMINING THE VOLATILE FATTY ACIDS OF CHEESE*

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Present-day knowledge regarding total volatile fatty acids of cheese, and particularly in regard to their interrelationship with cheese flavor, is relatively obscure. This lack of knowledge appears to be due, in part, to earlier unsatisfactory quantitative methods for determining these volatile acids.

Simple steam-distillation methods on cheese and other dairy products are now known to give low and variable results. This has been shown by various investigators (1, 4, 7, 8, 9). As a result of their observations on this subject, Hiscox, Harrison, and Wolf (5, 6, 7) developed an involved water-ether extraction method using steam distillation which recovered considerably more of the volatile fatty acids from cheese than did the simple steam-distillation method on cheese mush. Their method, however, has never been widely adopted in this country because of the elaborate and time-consuming technique required and the necessity of using special glass-joined stills of empirical dimensions.

Recently Smiley, Kosikowsky, and Dahlberg (9) published details of a simplified extraction-distillation method which possessed the advantages of being relatively quick, of not requiring special glass-joined stills, and of recovering fully as much of the volatile acids from cheese as the method of Hiscox, Harrison, and Wolf (7). Their method included the principles of ether extraction of an acid-cheese mixture, removal of volatile fatty acids from the ether by washing with dilute alkali, and acidification of the washings followed by direct distillation in the presence of $MgSO_4$. In the meantime, a specific amount of distilled water was added to the acid-residual material and the mixture directly distilled with $MgSO_4$. The sum of the titrations from these two distillations plus that of the alcohol rinse represented the total volatile fatty acidity of the cheese.

In the development of the above method for determining the volatile fatty acids of cheese it was discovered that under certain conditions direct distillation of cheese mush produced results which gave promise of further simplification of the technique.

Distillation of cheese mush, as is commonly used in simple steam distillation methods, fails to fully recover the volatile fatty acids of the cheese. Hiscox and Harrison (5) found this retention to be exercised by the fat and

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protein present, while the work of Smiley, Kosikowsky, and Dahlberg (9) showed that nonvolatile acids, such as palmitic and oleic, also exerted the same effect. It is, however, not improbable that the retentive effect of these cheese constituents could be greatly negated by means of more adaptable distillation techniques. Direct distillation without initial extraction has been used to estimate volatile fatty acids of various substances by a number of workers. Ferris, Redfield, and North (1) were able to recover more volatile fatty acids from butter by direct distillation than by simple steam distillation. Friedemann (2) used a direct distillation method in the determination of volatile fatty acids of biological materials with considerable success.

A successful development of a method involving rapid direct distillation of cheese mush with a minimum of retentive effect should prove to be of considerable importance in cheese or cheese-flavor research. The object of this paper is to present such a method.

EXPERIMENTAL

The rapid direct-distillation method. A 10-gram representative portion of cheese weighed on a torsion balance (sensitivity 4 mg.) was well ground in a mortar with warm 10 per cent sulphuric acid (50–55° C.). The ground cheese mixture was washed quantitatively from the mortar into an 800-ml. Kjeldahl flask with a total of 50-ml. of sulphuric acid solution, including both grinding and washing. A few glass beads and 35 grams of $MgSO_4 \cdot 7\ H_2O$ were added to the Kjeldahl flask. The entire mixture was well shaken and exactly 230 ml. of distilled water at 25° C. were added. Next this acid-cheese mixture was refluxed on a wire gauge square over a low flame for 3 to 5 minutes to drive off carbon dioxide. After slight cooling, exactly 20 ml. of freshly boiled distilled water at 25° C. were used to rinse down the refluxer. The flask was placed on a strongly supported asbestos board ¼ inch thick with a 3-inch opening on an ordinary Kjeldahl distillation apparatus, except that the traps were replaced by glass tubes of 10 mm. inside diameter to reduce refluxing to a minimum.

Distillation was begun with the distillate passing through slightly moistened coarse filter paper (S & S 595) of 11 cm. diameter and was continued until a total of 285 ml. distillate had been collected. In some instances it was not possible to collect 285 ml. In this event two other criteria of end point were used, crystallization and temperature reading of the distillation mixture. Crystallization occurred after intense foaming and was detected by a rapid thickening and settling out of the contents. In the case of temperature readings it was found that at the correct end point of the boiling mixture the temperatures ranged from 115° C. to 116° C. (corrected). The temperature should never go over 116° C. and the volume of distillate should not be lower than 280 ml., even with these additional criteria.

During distillation, and particularly near the end, the burners should be opened wide so as to allow a steady flame to cover as much of the exposed bottom surface of the flask as possible, but the flame should not be of such magnitude as to spread beyond this area. A low flame in which the tip or peak of the flame hits a limited area on the bottom of the flask should be avoided to prevent localized burning.

Burning of the contents can be detected by the appearance of a dark purplish discoloration at the surface of the bottom of the flask and can later be confirmed after removal of the flask by a characteristic burnt protein odor. Slight burning did not appear to affect results greatly, but even this ought to be avoided.

In order to accurately estimate the required amount of distillate, a 500-ml. Erlenmeyer flask marked to receive approximately 250 ml. was inserted under the condenser tube. When the distillate reached the 250-ml. mark, this flask was replaced by a 100-ml. graduate. The funnel for the insoluble fraction was also transferred to the graduate. While distillation continued, the distillate in the flask was titrated, then accurately measured in a 300-ml. graduate, and the amount of alkali used was subtracted. The difference between the volume of distillate and the required 285-ml. volume was collected in the 100-ml. graduate and titrated independently. The two titrations were grouped together as the water-soluble portion. Distillates were titrated with N/20 NaOH, using phenolphthalein as indicator. The final results, however, were presented according to standard form as ml. N/10 acid per 100 grams of cheese.

Neutral alcohol (25 ml.) was used to rinse the insoluble acids from the condenser through the filter paper and into a small Erlenmeyer flask. Alcohol rinsings were titrated in a manner similar to that employed with the water distillates. The sum of the titrations of the water distillates and of the alcohol rinse was considered the total volatile acidity of the cheese. Duplicates were run on all samples.

Analyses of cheeses by the rapid direct-distillation method and by other methods. The rapid direct-distillation method gave results surprisingly consistent with those obtained on the same cheeses by the method of Hiscox, Harrison, and Wolf (7) and also by the simplified extraction-distillation method of Smiley, Kosikowsky, and Dahlberg (9). Data illustrating this are presented in table 1. It may be noted in this table that 8 distinct types of cheese have been analyzed and that in each instance the rapid direct-distillation method gave results closely approaching those obtained by the other two methods.

As a further test of the effectiveness of this method a series of 60 Cheddar cheeses was analyzed for total volatile fatty acids by the rapid direct-distillation method and by the simplified extraction-distillation method. These cheeses included samples made from raw and pasteurized milk and ranged in age from 2 days to 3 years. Table 2 shows that there is very good agreement between these two methods on this series of Cheddar cheeses. The rapid direct-distillation method produced a slightly lower total average result for the 60 samples although the difference was within the experimental error of both methods. The average volatile fatty-acid content of the 60 cheeses was 30.1 ml. N/10 acid/100 grams cheese by the simplified extraction-distillation method, while by the rapid direct-distillation method the average value was 29.2 ml. N/10 acid/100 grams cheese or a total average difference of 0.9 ml. N/10 acid/100 grams cheese. On the average, approximately 10 per cent of the total volatile fatty acid value was due to insoluble volatile fatty acids.

TABLE 1

*The volatile acidity of eight different types of cheese as analyzed by the rapid direct-distillation method; the method of Hiscox, Harrison and Wolf; and the simplified extraction-distillation method**

Cheese	Hiscox *et al.*	Rapid direct-distillation	Simplified extraction-distillation
Blue	37.5	37.0	40.4
Switzer	47.1	48.0	49.5
Chantelle	29.9	33.4	31.4
Sharp ground cheese food	29.6	31.0	30.8
Trappist	51.4	47.9	49.2
Leiderkrantz	103.7	106.6	102.8
Camembert	39.0	38.8	39.9
Cheddar	60.6	61.7	61.8
Average	49.8	50.6	50.7

* All values expressed as ml. N/10 acid per 100 grams cheese.

The two methods are about equally accurate in producing checks between duplicate samples, although here again the simplified extraction-distillation method showed a smaller variation between duplicate samples than did the rapid direct-distillation method. For 60 cheeses, the average variation between duplicate samples was 1.32 ml. N/10 acid/100 grams cheese using the simplified-extraction method, whereas it was 1.41 ml. N/10 acid/100 grams cheese by the rapid direct-distillation method.

Recovery of volatile fatty acids from a mixture containing fat and protein. Since the rapid direct-distillation method gave results comparable to those produced by the simplified extraction-distillation method, it should recover most of the volatle fatty acids associated with cheese. This recovery is evidently accomplished in spite of the fact that fat and protein are present in the distillation mixture. In order to check this phase more fully, an experiment involving recovery of pure volatile fatty acids under conditions similar to the method was conducted. The volatile fatty acids used ranged from acetic to myristic. It has been shown that acids from butyric to lauric can be fully recovered from water using direct distillation with $MgSO_4$ (9).

Five ml. of N/10 volatile fatty acid were placed in a Kjeldahl flask and titrated with N/20 NaOH. Exactly 2.5 grams of ash-free casein and 3.0 grams of fresh butter oil were next added to the flask. This was followed by the addition of 35 grams of $MgSO_4$ and 270 ml. of distilled water. The mixture was well agitated and brought to pH 2 with a small amount of 50 per cent H_2SO_4 and then refluxed for 5 minutes. The refluxer was rinsed with 20 ml. of distilled water. Distillation of the mixture was begun on the slightly modified Kjeldahl apparatus and was continued until 285 ml. of distillate were recovered. This distillate and the neutral alcohol rinsings containing the insoluble volatile fatty acids were titrated with N/20 NaOH. After compensating for blanks the percentage recovery was calculated. These data are presented in table 3.

*The total volatile fatty acids of sixty Cheddar cheeses as determined by the rapid direct-distillation method and the simplified extraction-distillation method**

Cheese No.	Simplified extraction-distillation	Rapid direct-distillation	Cheese No.	Simplified extraction-distillation	Rapid direct-distillation
1	34.6	34.2	31	28.2	26.0
2	34.7	32.8	32	19.4	16.1
3	25.5	23.7	33	23.2	21.1
4	19.4	21.3	34	29.2	28.5
5	21.1	20.3	35	32.6	32.9
6	26.3	23.3	36	40.5	36.1
7	24.2	22.1	37	49.4	48.0
8	27.5	27.6	38	28.3	29.0
9	27.2	24.4	39	40.8	42.9
10	33.6	32.1	40	13.7	14.9
11	26.1	26.0	41	16.8	17.5
12	30.0	26.5	42	24.0	21.1
13	35.8	34.8	43	24.0	20.9
14	34.6	33.5	44	25.0	22.8
15	34.8	33.8	45	21.7	20.2
16	61.4	61.7	46	18.8	17.1
17	12.7	12.0	47	25.8	25.1
18	14.5	13.4	48	35.1	35.3
19	13.5	11.0	49	41.2	40.3
20	13.8	12.2	50	206.0	209.4
21	14.7	13.9	51	41.1	39.4
22	16.5	14.7	52	19.5	21.2
23	11.8	12.6	53	21.4	21.8
24	17.4	17.3	54	21.1	21.3
25	18.9	16.6	55	22.0	22.0
26	16.3	14.1	56	30.0	30.4
27	24.8	20.0	57	18.6	17.4
28	16.5	12.1	58	31.3	30.8
29	21.1	21.9	59	25.8	25.8
30	90.2	94.7	60	30.9	31.9
Average of 60 cheeses				30.1	29.2

* All values expressed as ml. N/10 acid per 100 grams cheese.

TABLE 3

The recovery of pure volatile fatty acids by direct distillation with $MgSO_4$ from a mixture containing fat and protein

Fatty acid	Recovered in presence of 2.5 grams casein and 3.0 grams butter oil
	per cent
Acetic (C_2)	98
Butyric (C_4)	97
Caproic (C_6)	97
Caprylic (C_8)	90
Capric (C_{10})	79
Lauric (C_{12})	46
Myristic (C_{14})	17

It is evident from this table that for the short-chain volatile fatty acids, such as acetic, butyric, and caprylic, recovery was close to 100 per cent, but for the long-chain volatile fatty acids a definite retentive effect was exerted by the butter fat and protein combination. The retentive effect was in direct relation to the number of carbon atoms in each fatty acid. As the short-chain fatty acids are the most important in cheese (10), the retentive effect should not prove to be of much consequence in regard to the method. These results approximated closely those obtained by Smiley, Kosikowsky, and Dahlberg (9) on the recovery of volatile fatty acids in the presence of palmitic and oleic acids under similar conditions of distillation.

The temperature of distillation. A reason for the more effective recovery of the volatile fatty acids by the new method would undoubtedly be the fact that a definite increase in the temperature of the boiling mixture

TABLE 4

The temperature of the distillation mixture and the gas phase above it at various periods in the distillation process and the resulting amount of volatile fatty acid recovered

Portions of distillate	Titration for volatile acids	Temperature in distillation mixture	Temperature in gaseous phase
ml.	*ml.**	*° C.*	*° C.*
1st drop over		102.0	99.8
50	7.5	102.7	99.8
50	6.8	102.9	99.8
50	6.8	103.5	99.9
50	7.0	104.0	99.9
50	8.3	106.0	100.0
10	2.3	107.2	100.0
10	2.5	109.5	100.0
10	2.5	113.0	100.5
5	1.5	115.7	100.8
Alcohol rinse	2.8	....	....
Total	47.8	....	....

* Ml. N/10 acid/100 grams cheese.

occurs during distillation. Friedemann (2) used the soluble salt $MgSO_4$ to raise the boiling point during direct distillation of volatile fatty acids in biological materials. The rapid direct-distillation method also required the use of $MgSO_4$.

Temperature changes occurring in the distillation mixture and the gaseous phase above it were observed during an actual analysis on a sample of cheese. From the first 250-ml. portion of distillate, 50-ml. quantities were collected independently and titrated, and at the same time the temperatures were noted at the time each portion was collected. At the very end of the distillation, portions were collected n 10-ml. and 5-ml. amounts.

It can be seen in table 4 that there was a gradual rise in the temperature of the boiling mixture during the distillation of the first 250-ml. portion. During the recovery of the last 35-ml. portion there was a rapid acceleration in temperature. The maximum temperature attained in the boiling mixture for this trial was 115.7° (corrected) while for the gaseous phase the maximum temperature was 100.8° C. (corrected). These maximum temperatures were maintained for about one minute. Other trials on various cheeses gave results of approximately the same magnitude.

The value of 47.8 ml. N/10 acid per 100 grams of cheese here was almost identical to the value obtained on the same cheese using the simplified extraction-distillation method.

Hydrolysis or decomposition of the cheese constituents under conditions of the method. The possibility that hydrolysis or decomposition of the cheese constituents might occur and result in the production of volatile substances was considered. Gould (3) has shown that heating skim milk in cans at 116° C. for 1- and 2-hour periods appreciably increased the volatile acid content of the milk as determined by steam distillation. Lactose by its subsequent destruction was considered as being the compound most responsible for this increase.

As most ripened cheeses usually have lost all the lactose by bacterial fermentation in a few days after making, the findings of Gould (3) apparently are not applicable in this case. Gould (3), also maintained his temperature of 116° C. for a much longer period than was required by our method. Nevertheless, an investigation was conducted to note if any hydrolysis or decomposition occurred under conditions of the method.

Specific quantities of fresh butter oil, purified casein, and lactose in various combinations were mixed with 50 ml. of 10 per cent H_2SO_4 brought up to the desired amount with water, followed by the distillation procedure outlined for the method. At the same time the steam distillation technique of Hiscox, Harrison, and Wolf (7) was also applied.

Table 5 shows the effect of the distillation procedure on fat, and fat and casein. The distillate was titrated in small portions in order to determine if definite increases in volatile acidity occur near the end of the distillation where the temperature had increased. The fact that no exaggerated increases appeared at this point and that all the blanks were low and checked well with those obtained by the method of Hiscox, Harrison, and Wolf (7), where lower temperatures of distillation were used and where fat and casein were not involved, pointed to the conclusion that no apparent hydrolysis or decomposition occurs during the operation of this method.

When pure lactose was used alone or in combination with casein, a small blank was also obtained. The blank obtained for the distillate from 5 grams of lactose was 0.12 ml. N/10 acid, and when 5 grams of lactose were mixed with 2.5 grams of casein the resulting titration for the distillate was only 0.40 ml. N/10 acid. In view of the fact that distillation with plain water usually gave a blank value of 0.10 ml. N/10 acid, this was a rather good indication that lactose, either alone or in combination with protein, was not greatly affected by the temperature under the conditions of the method.

TABLE 5

The titration blanks obtained on fresh butter oil and ash-free casein using the rapid direct-distillation technique and the extraction method of Hiscox, Harrison, and Wolf (7)

2.5 gm. casein + 4.4 gm. butter oil		4.4 gm. butter oil	
Distillate portions	Titration	Distillate portions	Titration
ml.	*ml. N/10 acid**	*ml.*	*ml. N/10 acid*
	Rapid direct distillation method		
50	0.03	50	0.03
50	0.03	50	0.03
50	0.03	50	0.03
50	0.06	50	0.04
50	0.08	50	0.03
10	0.03	10	0.03
10	0.03	10	0.04
10	0.03	10	0.03
10	0.03	10	0.03
3	0.02	6	0.04
Alcohol rinse	0.06	Alcohol rinse	0.07
Total	0.43		0.37
	Method of Hiscox, Harrison, and Wolf		
Total ...	0.40		0.28

* 0.03 ml. represented 1 drop from burette.

DISCUSSION

The rapid direct-distillation method presented here possesses several distinct advantages. It recovers fully as much of the volatile fatty acids of cheese as are recovered by the method of Hiscox, Harrison, and Wolf (7) or by the simplified extraction-distillation method (9). This is accomplished without the use of special glass-stills and with a minimum of analytical apparatus and chemical reagents. The new method also possesses the advantage of being very timesaving. This is more clearly illustrated by the fact that the method of Hiscox, Harrison, and Wolf (7) requires approximately 40 hours to complete an individual analysis; that by the simplified-extraction method of Smiley, Kosikowsky, and Dahlberg (9) about 4 hours are required; while by the rapid direct-distillation method this time requirement is reduced to approximately 80 minutes.

It is not believed that this method should supplant the simplified extraction-distillation method at all times, for there may be circumstances when ether extraction would be desirable. Also, although the rapid-distillation method appears quite dependable from the standpoint of producing duplicate results, the simplified-extraction method appears from a comparative study of available data to be slightly more accurate and consequently could be used as a check method. Although greater care is required to reach a proper end point by the rapid direct-distillation method, it appears very well suited to a situation where a large number of cheese samples are to be analyzed over a short period of time with a good degree of accuracy.

Initially in the development of the method ortho phosphoric acid was first used as the solvent but it became apparent after a series of analyses that for some reason recovery was not so complete as that obtained by the simplified extraction-distillation method. When 10 per cent H_2SO_4 was substituted for 10 per cent H_3PO_4 more complete recovery was attained and the results checked well with those obtained by the simplified extraction-distillation method. As a cheese solvent, 10 per cent H_2SO_4 appeared to be as effective as 10 per cent H_3PO_4.

Direct distillation under conditions of the new method recovered quite completely the lower-chain volatile fatty acids from a mixture of fat and protein. As these acids are the ones mostly associated with ripened cheeses this high percentage of recovery emphasizes the fact that the method should produce results comparable to other standard methods. The reason for this effective recovery is probably largely due to the higher boiling point reached in the distillation mixture of higher specific gravity.

There was no evidence that partial hydrolysis or decomposition occurred in the mixture during distillation under conditions of the method.

The precautions necessary to this method require emphasis. In weighing out samples for a test the cheese has to be cut up into small particles and be well mixed. Duplicate samples should always be taken from the same pile of well-mixed cheese.

In this method distillation should normally proceed until complete crystallization of the contents occurs. However, due to the turbid nature of the mixture, it is quite difficult to accurately determine crystallization as an end point. Consequently the use of a measured volume of distillate (285 ml.) is recommended. This amount of distillate under normal circumstances should carry the distillation mixture very close to complete crystallization. However, in a few instances it may not be possible to collect exactly this amount before crystallization occurs, and, if distillation is carried further in the attempt to collect the desired amount, burning will follow which may result in higher results. In this case crystallization of the contents or temperature reading may be employed as a criterion of end point. Crystallization usually follows a period of intense foaming and is detected by a rapid thickening and settling of the distillation mixture. Burners should be turned off immediately at this point. The temperature of the distillation mixture at the correct end point ranges from 115 to 116° C. (corrected). Temperatures over 116° C. should be avoided. Even with these two additional criteria the amount of distillate collected should not be less than 280 ml.

A steady full flame directed so as to cover the general area of the exposed bottom surface of the flask is required. Application of a low flame so as to contact a very limited area of the flask bottom should be avoided as this seems to encourage local burning near the very end of distillation. However, at no time should the flame be allowed to go beyond the bottom area of the flask limited by the 3-inch asbestos board opening. Tight fittings of the flasks in their asbestos sockets are necessary.

The distillation condenser tubes should be washed periodically with neutral alcohol and distilled water, and the alcohol should be checked frequently for neutrality.

SUMMARY

A very short and rapid method for the determination of volatile fatty acids in cheese is presented. This new method required that a finely ground sulphuric acid–cheese mixture with a low pH be refluxed to drive off CO_2 and directly distilled in the presence of $MgSO_4$. A specific quantity of water (250 ml.) was added prior to distillation and the amount of distillate recovered was limited to 285 ml. The insoluble volatile fatty acids were washed from the condenser tube by neutral alcohol. Distillates were titrated with N/20 NaOH, using phenolphthalein as indicator. The sum of the titrations of the water distillate and the alcohol rinse represented the total volatile acidity of cheese.

The advantages of this method are that high recovery of the volatile fatty acids from cheese is accomplished in a very short time without the use of special glass-joined stills and with a minimum of apparatus and chemical reagents. This method is very adaptable to circumstances where large numbers of cheeses need to be tested in a short time.

Direct distillation in the presence of $MgSO_4$ brought about a very effective recovery of pure lower-chain fatty acids when fresh fat and casein were in the mixture. Under these conditions, recovery was indirectly related to the number of carbon atoms present in each fatty acid.

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AUTHOR INDEX OF ORIGINAL ARTICLES

ALBRECTSON, RAYMOND, training inseminators, 482

ALLEN, N. N., effect of ingestion of d.d.t., 530; variations in weight of dairy cows, 544

ALMQUIST, JOHN O., dyes used for semen identification, 554; semen identification, 815

AMERICAN DAIRY SCIENCE ASSOCIATION, announcement, 63; officers and committees, 64; announcement, 129

ARBUCKLE, W. S., salt determination in butter and new cheese, 431

ARNOLD, P. T. DIX, effects of stilbestrol, 524

ARRINGTON, L. R., growth and blood composition of dairy calves, 527

ASHWORTH, U. S., determining the solubility of milk powders, 485

ATKESON, F. W., vitamin A and carotene content of butter, 91; vitamin A in the blood and liver of calves, 515

BABCOCK, C. J., frozen homogenized milk, 699

BABEL, F. J., cultures to bacteriophage, 496; acid production by cheese cultures, 589; acid production by cheese cultures, 597

BAKER, M. P., the direct microscopic count, 506

BARRETT, G. R., pregnancy diagnosis, 553; time of insemination and conception rate, 556

BATE, WILBUR, influence of homogenization of fat on haircoat of dairy calves, 41

BEACHBOARD, RUSSELL, activity of cheese cultures, 498

BEATY, ANNABEL, nonfat dry milk solids in bread, 511; dry milk in bread, 821

BECKER, R. B., evaluation of pasture, 474; effects of stilbestrol, 524; shark meal as a protein supplement, 536; shark meal as a protein supplement, 801

BELL, R. W., physical stability of frozen cream, 213

BENDER, C. B., calcium metabolism in lactating dairy cows, 538

BENNETT, EMMETT, bactericidal properties of surface-active agents, 751; "quality" in hays, 795

BORTREE, A. L., factors influencing rumen microflora, 542; storage and treatment of milking machine, 849

BOWLING, G. A., purebred dairy cattle association, 479

BRANTON, CECIL, estimation of the weight of bulls, 141

BUCKNER, CECILIA R., bovine mastitis, 528

BURGWALD, L. H., effect of route delivery on milk, 273

BURKEY, L. A., isolation of whey proteins, 503; bovine mastitis, 528

CALDWELL, M. J., vitamin A in the blood and liver of calves, 515; rumenectomized calf, 543

CANNON, C. Y., influence of homogenization of fat on haircoat of dairy calves, 41

CARY, C. A., unidentified nutrient in roughages and in milk, 549

CASIDA, L. E., pregnancy diagnosis, 553; time of insemination and conception rate, 556

CAVE, H. W., ground mungbeans as a protein supplement, 537

CHINN, STANLEY H. F., non-lactose fermenting yeasts from butter, 507

CHOI, R. P., moisture in dry products, 639; determination of ammonia, 645

CHRISTIANSEN, C. V., quality symposium, 494

CLARK, ESTHER L., differential nitrogen retention, 717

COLE, WILFORD C., heat coagulations of milk, 421

COMBS, W. B., dry ice cream mix in storage, 488

CONE, FRANK J., determining the solubility of milk powders, 485

CONVERSE, H. T., effect of vitamin A deficiency on reproduction, 522; effect of vitamin A deficiency on bulls, 669

Copeland, Lynn, feed cost of milk production, 345

Coulter, S. T., dry ice cream mix in storage, 488; fluorescence of dry whole milk, 491; reducing materials in dry whole milk, 492; drying milk, 492

Crisler, O. S., treating mastitis, 529

Crowe, L. K., reducing materials in dry whole milk, 492

DAHLBERG, A. C., occurrence and survival of *Brucella abortus*, 71; determination of the volatile fatty acids of cheese, 307; quality symposium, 494; coliform bacteria in pasteurized milk, 508; coliform bacteria in pasteurized milk, 651; determining volatile fatty acids, 861

Dahle, C. D., a grating type cheese, 498

Davis, G. K., shark meal as a protein supplement, 536; shark meal as a protein supplement, 801

Davis, H. P., feeding synthetic thyroprotein, 534

Davis, R. N., effect of cottonseed in the ration, 839; Babcock vs. Mojonnier test, 845

Dexter, S. T., lactation factors in corn silage, 542

Deysher, Edgar F., the keeping quality of samples of commercially dried milk packed in air and in inert gas, 55; storage life of evaporated milk, 499

Dinwiddie, W. K., central artificial breeding organizations, 552

Doan, F. J., evaporated milk, 625

Duncan, C. W., lactation factors in corn silage, 542

Dunlap, William A., frozen homogenized milk, 699

Dunn, K. M., factors influencing rumen microflora, 542

EATON, H. D., supplementary vitamins for calves, 231

Ellertson, M. E., effect of heat on milk dialysates, 727

Ely, Fordyce, clipping vs. grazing in estimating yield of pastures, 393; evaluation of bluegrass pasture, 540; factors influencing rumen microflora, 542

Espe, Dwight, influence of homogenization of fat on haircoat of dairy calves, 41

Ewing, John, feeding barn dried hay, 547

FAIRBANKS, B. W., moisture in dry products, 639; determination of ammonia, 645

Forty-first annual meeting, 321

Fouts, E. L., effectiveness of dairy detergents, 512

Frantz, R. S., formic acid content of milk heated to high temperatures as determined by the distillation procedure, 27; lactic acid in dairy products. IV. effects of storage of evaporated milk on lactic and formic acid production, on pH, and on acidity and formol titrations, 33

Freeman, Theo. R., acid inversion of sucrose, 101; effectiveness of dairy detergents, 512

Fuller, James E., bactericidal properties of surface active agents, 751

GAINES, W. L., live weight versus metabolic body size, 259

Gallup, W. D., ground mungbeans as a protein supplement, 537

Garrett, O. F., quality symposium, 494

Gilman, H. L., occurrence and survival of *Brucella abortus*, 71

Gleim, Wm., effect of heat on milk dialysates, 727

Golding, N. S., gas packing cheese, 495; action starter organisms, 495

Gould, I. A., formic acid content of milk heated to high temperatures as determined by the distillation procedure, 27; lactic acid in dairy products. IV. effects of storage of evaporated milk on lactic and formic acid production, on pH, and on acidity and formol titrations, 33; variations in the acid degree of milk fat, 504; concentration of the free fatty acid portion of milk, 505

Greenbank, George R., the keeping quality of samples of commercially dried milk packed in air and in inert gas, 55; rate of autoxidation of milk fat, 145

Gregory, P. W., deleterious recessive genes, 557

GULLICKSON, T. W., defluorinated rock phosphate as a phosphorus supplement, 317

GUTHRIE, E. S., tallowy flavor in milk, 293; effect of feeding cod-liver oil on flavors and vitamin C, 349; results of deaeration of milk, 359

HAAG, J. R., relation of carotene levels to fertility, 522; utilization of non-protein nitrogen, 535

HALL, S. R., effect of vitamin A deficiency on reproduction, 522; effect of vitamin A deficiency on bulls, 669

HANKINSON, C. L., effect of heat on milk dialysates, 727

HANSEN, R. G., effect of vitamin supplements on calves, 517; vitamin A and globulin content of colostrum, 521; vitamin supplements for calves, 761; vitamin A in colostrum, 809

HARLAND, F. G., effect of cottonseed in the ration, 839; Babcock vs. Mojonnier test, 845

HARTMAN, A. M., unidentified nutrient in roughages and in milk, 549

HEINEMANN, BURDET, testing 80 per cent cream, 689

HENDERSON, H. O., effect of pregnancy on body weight, 657

HENDERSON, J. L., a water-soluble antioxidant, 501

HENKE, L. A., use of urea in rations, 629

HERMAN, H. A., treating mastitis, 529; central artificial breeding organizations, 552

HERREID, E. O., sanitizing the centrifugal separator, 513; "in-training" course for dairy manufactures, 513

HERRINGTON, B. L., calculation of total solids by Sharp and Hart equation, 87

HETRICK, J. H., relative keeping qualities of processed milks, 489

HIBBS, J. W., blood picture of dairy cows at parturition, 514; effect of feeding vitamin A on calves, 519; studies on milk fever, 617; milk fever in dairy cows, 617

HIGUCHI, KIYOSHI, relation of silage to quality of cheese, 157; carotene and vitamin A contents of cheese, 789

HILTON, J. H., loss of nutrients in hay and silage, 239

HINTON, S. A., feeding barn dried hay, 547

HODGSON, R. E., loss of nutrients in hay and silage, 239; effect of vitamin A deficiency on reproduction, 522; harvesting and preserving forage crops, 547; wilted alfalfa silage and alfalfa hay, 551; effect of vitamin A deficiency on bulls, 669

HOLM, GEORGE E., the keeping quality of samples of commercially dried milk packed in air and in inert gas, 55; rate of autoxidation of milk fat, 145; oxygen content of dried milk, 207

HOLMES, ARTHUR D., vitamin content of mare's milk, 163

HORWOOD, RUSSELL E., use of herd records, 476

HOSTERMAN, W. H., harvesting and preserving forage crops, 547

HOWE, JOHN W., crossing zebu with cattle in Jamaica, 552

HUFFMAN, C. F., lactation factors in corn silage, 542; factors influencing rumen microflora, 542

HUFNAGEL, C. F., heat-stability and viscosity of cream-style foods, 221; storage life of evaporated milk, 499; sterilized caramel milk, 607

HUGHES, J. S., vitamin A and carotene content of butter, 91; vitamin A in the blood and liver of calves, 515

HUNZIKER, O. F., drying milk, 485

HUSSONG, R. V., quality symposium, 494; sanitizing the centrifugal separator, 513

HYATT, GEO., JR., effect of feeding iodinated casein, 533

JACK, E. L., biochemical changes in powders, 486

JENNESS, R., fluorescence of dry whole milk, 491; reducing materials in dry whole milk, 492

JENSEN, C., sanitization of creamery water supplies, 510

JENSEN, J. M., measuring detergency functions, 453; storage and treatment of milking machine, 849

JEZESKI, J. J., cryophilic organisms in water and butter, 439

JOHNSON, ARNOLD H., nonfat dry milk solids in bread, 511; dry milk in bread, 821

Johnson, B. C., variations in the acid degree of milk fat, 504
Johnston, Floyd, uniform rules for official testing, 480
Jones, I. R., relation of carotene levels to fertility, 522; utilization of non-protein nitrogen, 535
Josephson, D. V., effect of route delivery on milk, 273; judgment of flavor defects, 500; effect of light on riboflavin content and flavor of milk, 508; evaporated milk, 625
Judkins, H. F., quality symposium, 494

Kaeser, H. E., some physiological effects of extending the colostrum feeding period of dairy calves, 13; colostrum in calf feeding, 517
Keener, H. A., composition of calf blood, 663
Kendrick, J. F., dairy herd improvement association program, 473
King, W. A., loss of nutrients in hay and silage, 239
Knodt, C. B., carbohydrate metabolism of mammary gland tissue in vitro, 115; studies of the carbohydrate metabolism of mammary gland tissue in vitro, 121; effect of sulfanilamide upon spermatozoa, 285
Knoop, C. E., silage from drought-damaged corn, 541; effect of feeding potassium iodide and skim milk powder, 555
Kosikowsky, F. V., determination of the volatile fatty acids of cheese, 307; determining volatile fatty acids, 861
Krauss, W. E., loss of nutrients in hay and silage, 239; effect of feeding vitamin A on calves, 519; ground ear corn as compared to ground shelled corn, 539; studies on milk fever, 617; milk fever in dairy cows, 767
Krienke, W. A., brown discoloration of powdered whole milk, 488; relative keeping qualities of processed milks, 489
Krukovsky, Vladimir N., tallowy flavor in milk, 293
Kuhlman, A. H., ground mungbeans as a protein supplement, 537
Kunkel, R. W., dry ice cream mix in storage, 488
Kuzmeski, J. W., quality in hays, 795

Lardy, H. A., effect of ingestion of D.D.T., 530
Larson, R., fluorescence of dry whole milk, 491
Lindquist, Harry G., vitamin content of mare's milk, 163
Link, R. P., rumenectomized calf, 543
Lloyd, C. A., pregnancy diagnosis, 553
Loosli, J. K., supplementary vitamins for calves, 231; carotene utilization in the newborn calf, 381; placental transmission of vitamin A, 516; placental transmission of vitamin A, 707
Lush, J. L., artificial insemination and genetic improvement, 557

Macy, H., agent responsible for effect of propionates, 173; cryophilic organisms in water and butter, 439
Mann, A. I., technique for intravenous feeding of ruminants, 526
Marquardt, J. C., occurrence and survival of *Brucella abortus*, 71
Marshall, Sidney P., effects of stilbestrol, 524; shark meal as a protein supplement, 536; shark meal as a protein supplement, 801
Martin, W. H., vitamin A and carotene content of butter, 91
Maruyama, C., use of urea in rations, 629
McKey, Beula V., vitamin content of mare's milk, 163
Mead, S. W., deleterious recessive genes, 557
Mercier, Ernest, seasonal effect on spermatogenic activity, 556
Miller, G. D., grazing performance of cows, 199; factors influencing respiration rate, body temperature and pulse rate of dairy cows, 465; effect of humidity and temperature, 544; kobe lespedeza hay, 613
Monroe, C. F., loss of nutrients in hay and silage, 239; ground ear corn as compared to ground shelled corn, 539; silage from drought-damaged corn, 541; studies on milk fever, 617; milk fever in dairy cows, 767
Monroe, R. A., thyroid secretion rate, 581
Moore, L. A., effects of feeding thyroprotein, 532

MORRISON, H. B., clipping vs. grazing in estimating yield of pastures, 393; evaluation of bluegrass pasture, 540

MORROW, K. S., composition of calf blood, 663

MUELLER, W. S., bactericidal properties of surface-active agents, 751

NAGEOTTE, J. C., calf club work in Pennsylvania, 483

NELSON, F. E., non-lactose fermenting yeasts from butter, 507

NORTON, C. L., supplementary vitamins for calves, 231; carotene utilization in the newborn calf, 381; value of hays for young dairy cows, 546; nutritive value of barn-cured hay, 549

NYSTROM, AMER B., quality improvement, 484

OLSON, FLOYD C., defluorinated rock phosphate as a phosphorus supplement, 317

OLSON, H. C., activity of cheese cultures, 498

OLSON, J. C., JR., agent responsible for effect of propionates, 173

O'MALLEY, C. M., moisture in dry products, 639; determination of ammonia, 645

PARKHURST, RAYMOND T., feed ingredient research, 539

PARKINSON, LEONARD R., vitamin content of mare's milk, 163

PARRISH, D. B., vitamin A and carotene content of butter, 91

PERKINS, A. E., silage from drought-damaged corn, 541; fan-ventilated long hay, 550

PERRY, E. J., management for artificial breeding associations, 481

PETERSEN, W. E., the effect of increasing the negative pressure and widening of the vacuum-released ration on the rate of removal of milk from the udder, 45; carbohydrate metabolism of mammary gland tissue in vitro, 115; studies of the carbohydrate metabolism of mammary gland tissue in vitro, 121

PETERSEN, W. H., relation of silage to quality of cheese, 157; pantothenic acid, niacin and biotin content of milks, 783; carotene and vitamin A contents of cheese, 789

PFAU, K. O., calcium metabolism in lactating dairy cows, 538

PHILLIPS, PAUL H., preservation of bull semen, 407; effect of vitamin supplements on calves, 517; vitamin A and globulin content of colostrum, 521; vitamin supplements for calves, 761; vitamin A in colostrum, 809

PIERCY, P. L., blood studies of dairy cows, 526; blood studies on cows, 831

POUNDEN, W. D., milk fever in dairy cows, 767

PRICE, W. V., relation of silage to quality of cheese, 157; quality symposium, 494

PROUTY, C. C., gas packing cheese, 495; action of starter organisms, 495

PUTNAM, DEXTER N., effect of pregnancy on the body weight, 657

PYENSON, HARRY, a spectrophotometric study of the changes in peroxide value of spray-dried whole milk powder during storage, 1; keeping quality of spray dried ice cream mix, 371; keeping quality of spray-dried ice cream mix, 493; manufacture of powdered cream-mix, 511

RANDALL, RAYMOND, frozen homogenized milk, 699

REAVES, P. M., growth and blood composition of dairy calves, 527

REECE, RALPH P., influence of a synthetic thyroprotein, 533

REGAN, M. J., central artificial breeding organizations, 552

REGAN, W. M., deleterious recessive genes, 557

REID, J. T., calcium metabolism in lactating dairy cows, 538

REYNOLDS, J. N., gas packing cheese, 495

RIGGS, LLOYD K., nonfat dry milk solids in bread, 511; dry milk in bread, 821

ROERIG, RICHARD N., frozen homogenized milk, 699

RUPEL, I. W., effect of vitamin supplements on calves, 517; vitamin supplements for calves, 761

RUSOFF, L. L., blood studies of dairy cows, 526; kobe lespedeza hay, 613; blood studies on cows, 831

SAGER, OSCAR S., improvements in the phosphatase test, 507; the phosphatase test as applied to cheese, 737

SALISBURY, G. W., estimation of the weight of bulls, 141; effect of sulfanilamide upon spermatozoa, 285; influence of age on breeding efficiency, 337; calcium metabolism in lactating dairy cows, 538; fertility of bull semen, 695

SANDERS, C. F., physical stability of frozen cream, 213

SANDERS, G. P., ripening of pasteurized-milk cheddar cheese, 497; improvements in the phosphatase test, 507; the phosphatase test as applied to cheese, 737

SCHAFFER, P. S., rate of autoxidation of milk fat, 145; oxygen content of dried milk, 207

SCHEIDENHELM, E. C., program suggestions for inter-breed groups, 480

SCHNAUTZ, J. O., relation of carotene levels to fertility, 522

SCHOENLEBER, L. G., harvesting and preserving forage crops, 547

SCHREITER, O. M., reconstitutability of dry whole milk, 490

SCHULTZE, A. B., feeding synthetic thyroprotein, 534

SCOTT, E. C., quality symposium, 494

SEATH, D. M., grazing performance of cows, 199; factors influencing respiration rate, body temperature and pulse rate of dairy cows, 465; effect of humidity and temperature, 544; kobe lespedeza hay, 613

SHADWICK, G. W., quality symposium, 494

SHAW, J. C., ketosis in dairy cattle, 131; ketosis in dairy cattle, 151; composition of mammary venous bloods of cows, 183; ketosis in dairy cows, 523; technique for intravenous feeding of ruminants, 526

SHEPHERD, J. B., harvesting and preserving forage crops, 547; wilted alfalfa silage and alfalfa hay, 551

SKINNER, LOUISE, silage from drought-damaged corn, 541; fan-ventilated long hay, 550

SMILEY, K. L., determination of the volatile fatty acids of cheese, 307

SMITH, VEARL R., the effect of increasing the negative pressure and widening of the vacuum-release ration on the rate of removal of milk from the udder, 45; vitamin A and globulin content of colostrum, 521; blood serum calcium of cows at parturition, 528; vitamin A in colostrum, 809

SPIELMAN, A. A., supplementary vitamins for calves, 231; carotene utilization in the newborn calf, 381; placental transmission of vitamin A, 516; vitamin A content of colostrum, 520; placental transmission of vitamin A, 707

SPITZER, ROBERT R., preservation of bull semen, 407

STABILE, JOSEPH N., frozen homogenized milk, 699

STALLARD, J. E., county testing laboratories, 474

STEFANIAK, J. J., pantothenic acid, niacin and biotin content of milks, 783

STOLTZ, R. B., effect of route delivery on milk, 273; the forty-first annual meeting of the American Dairy Science Association, 559

SUPPLEE, G. C., differential nitrogen retention, 717

SUTTON, T. S., some physiological effects of extending the colostrum feeding period of dairy calves, 13; colostrum in calf feeding, 517; studies on milk fever, 617; milk fever in dairy cows, 767

SWEETMAN, W. J., effect of vitamin A deficiency on reproduction, 522; wilted alfalfa silage and alfalfa hay, 551; effect of vitamin A deficiency on bulls, 669

SWETT, W. W., bovine mastitis, 528

TANABE, T., influence of age on breeding efficiency, 337

TARASSUK, N. P., heat coagulations of milk, 421; biochemical changes in powders, 486; a water-soluble antioxidant, 501

TEERI, A. E., composition of calf blood, 663

THOMAS, J. W., carotene utilization in the newborn calf, 381; placental transmission of vitamin A, 516; vitamin A content of colostrum, 520; placental transmission of vitamin A, 707

THOMPSON, W. W., rumenectomized calf, 543

Tittsler, Ralph P., ripening of pasteurized-milk cheddar cheese, 497

Tracy, P. H., a spectrophotometric study of the changes in peroxide value of spray-dried whole milk powder during storage, 1; keeping quality of spray-dried ice cream mix, 371; brown discoloration of powdered whole milk, 488; relative keeping qualities of processed milks, 489; reconstitutability of dry whole milk, 490; keeping quality of spray-dried ice cream, 493; quality symposium, 494; manufacture of powdered cream-mix, 511; sanitizing the centrifugal separator, 513; "in-training" course for dairy manufactures, 513

Trout, G. M., time required for making flavor judgments, 415; quality symposium, 494

Turk, K. L., carotene utilization in the newborn calf, 381; vitamin A content of colostrum, 520; nutritive value of barn-cured hay, 549; placental transmission of vitamin A, 707

Turner, C. W., elimination of male hormone by cows, 525; thyroid secretion rate, 531

Tysdal, H. M., harvesting and preserving forage crops, 547

VANLANDINGHAM, A. H., effect of feeding iodinated casein, 533

WALTER, HOMER E., ripening of pasteurized-milk cheddar cheese, 497; isolation of whey proteins, 503

Washburn, R. G., fan-ventilated long hay, 550

Watrous, G. H., Jr., a grating type cheese, 498

Weakley, Chas. E., Jr., effect of feeding iodinated casein, 533

Weaver, Earl, lactic acid in dairy products, IV. effects of storage of evaporated milk on lactic and formic acid production, on pH, and on acidity and formal titrations, 33

Webb, B. H., heat-stability and viscosity of cream-style foods, 221; storage life of evaporated milk, 499; sterilized caramel milk, 607

Wertz, Anne W., vitamin content of mare's milk, 163

Willard, H. S., hay palatability trials, 546

Willett, E. L., use of urea in rations, 629

Williams, J. W., vitamin A and globulin content of colostrum, 521

Wilson, H. F., effect of ingestion of d.d.t., 530

Wilson, H. L., quality symposium, 494

Wilster, G. H., reconstitutability of dry whole milk, 490

Wise, G. H., vitamin A in the blood and liver of calves, 515; rumenectomized calf, 543

Wiseman, H. G., effect of vitamin A deficiency on reproduction, 522; effect of vitamin A deficiency on bulls, 669

Wright, Philip A., the keeping quality of samples of commercially dried milk packed in air and in inert gas, 55

Wylie, C. E., feeding barn dried hay, 547

SUBJECT INDEX OF ORIGINAL ARTICLES

ACETONE bodies,
  in blood of cows, 183
Acid,
  amino, 183
  ascorbic, 625
  effect on tallowy flavor, 293
  betahydroxybutyric, 115
  citric,
    mammary gland metabolism, 115
  degree of milk fat, 504
  free, 505
  formic in milk, 27, 33
  inversion of sucrose, 101
  lactic,
    effect of storage on, 33
  production by cheese cultures, 589, 597
  pyruvic, 183
Adrenal gland, 523
Alfalfa,
  hay, 546, 547, 549, 550, 551
  silage
Alpha lactose,
  dehydration, 639
American Dairy Science Association,
  announcements, 63, 129, 257
  annual meeting proceedings, 559
  annual meeting program, 321
  officers and committees, 64
Ammonia, determination, 645
Antioxidants, 501
Artificial insemination, 285, 337, 407, 481, 482, 552, 553, 554, 555, 556, 557, 695, 815

BACTERIA,
  Brucella abortus in cheese, 71
  coliform in milk, 651
  counts, 506
  cryophilic in water and butter, 439
Bacteriophage, 496, 597
Blood composition, 183, 514, 515, 526, 527, 528, 538, 663, 831
Bluegrass pasture,
  yield estimation, 393, 540
Bread,
  milk solids in, 511
Breeding dairy cattle, 552, 557
Breeding efficiency, 337
Breeds of cattle,
  efficiency of, 345
Brucella abortus,
  survival in cheese, 71
Bulls,
  effect of iodine feeding on, 555
  effect of thyroprotein feeding on, 534
  estimating weight of, 141
  semen dilution, 695
  vitamin requirements for reproduction, 522, 669
Butter,
  mold inhibitors, 173
  salt determination, 431
  vitamin A potency of, 91
  yeasts from, 507

CALCIUM,
  blood level, 526, 528
  metabolism, 538
Calf clubs, 483
Calves, dairy,
  blood composition, 13, 515, 517, 519, 527, 663
  carotene utilization by, 381
  effect of homogenized milk on, 41
  feeding, 13, 517
  vitamin A and carotene in blood of, 13, 515, 517, 519, 707
  vitamin supplements for, 231
Caramel milk,
  manufacture of, 607
Carotene,
  in calf blood, 13, 515, 517, 519
  utilization by calves, 381
Cheese,
  Cheddar,
    acid development in, 496, 589, 597
    Brucella abortus survival in, 71
    cultures, 495, 498, 589, 597
    manufacture from pasteurized milk, 497
  phosphatase test on, 507, 737
    quality, 157, 495
  cottage, 597
  gas packing, 495
  grating type, 498
  salt determination, 431

  vitamin content of, 789
  volatile fatty acids in, 307, 861
Cleaning equipment, 453, 512, 751
Coliform bacteria, 651
Colostrum, 13, 515, 517, 520, 521, 809
Copper,
  relation to tallowy flavor, 293
Corn for dairy cows, 539
Cottonseed meal, 839
Cows, dairy,
  body temperature, 465
  blood composition, 183, 526
  ear corn versus shelled corn for, 539
  effect of age on breeding efficiency, 337
  effect of feeding vitamin D on, 514
  live weight versus metabolic body size, 259
  phosphorus supplements for, 317
  use of urea by, A629
  weight, 657
Cream,
  powdered mix, 511
  separator cleaning, 513
  testing, 689
Creamery water supplies, 510
Cultures,
  cheddar cheese, 495, 498, 589, 597

DAIRY,
  bulls,
    effect of iodine on, 555
    effect of thyroprotein on, 534
    reproductive performance, 522
  calves,
    blood composition, 13, 515, 517, 519, 527
    carotene utilization by, 381
    effect of homogenized milk on, 41
    feeding, 13, 517
    vitamin supplements for, 231, 761
  cattle,
    breeding, 552, 553
  cows,
    blood composition, 831
    body temperature, 465
    ear corn vs. shelled corn for, 539
    effect of environmental temperatures on, 544
    effect of iodinated casein on, 532, 533
    effect of vitamin D on, 514
    pulse rate, 465
    respiration rate, 465
    weight variation, 544
  products,
    antioxidants for, 501
    judging, 500
    moisture studies, 639
    quality symposium, 494
D.D.T.,
  effect on cows, 530
Detergency measurement, 453
Detergents, 453, 412
Dialysates of milk,
  effect of heat on, 727
Direct microscopic counts, 506
Disease,
  Brucellosis, 71
  ketosis, 131, 151, 183, 523
  mastitis, 528, 529
  milk fever, 617, 767
Dry milk,
  ammonia determination, 645
  brown discoloration, 488
  fluorescence, 491
  keeping quality, 486, 488, 489, 492
  peroxide value, 1
  solubility, 485, 490
  use in bread, 511, 821
Drying milk, 485, 492

EVAPORATED milk,
  stability of ascorbic acid in, 625
  storage life, 499

FAT,
  acid degree of, 504
  effect of cottonseed feeding on, 839
  oxidation, 145
  testing of cream, 689
Fatty acids,
  free, 505
  volatile, determination of, 307, 861
Feeds,
  corn, 539
  cottonseed meal, 839
  hay, 346, 547, 549, 550, 551, 613
  pastures, 393, 540
  shark meal, 536, 801
  silage, 157, 541, 542
  research, 539
Flavor,
  defects, 501

judging, 500
tallowy, 293
Foods,
cream style, 221
from whey proteins, 503
Formic acid determination, 27
Formol titration,
effect of storage on, 33
Frozen milk,
homogenized, 699
keeping quality, 489

GLOBULIN,
content of colostrum and blood, 521
Goats,
live weight vs. metabolic body size, 259
Grating type cheese, 498

HAY, 346, 547, 549, 550, 551
Kobe lespedeza, 613
loss of nutrients from, 239
quality of, 795
Heat coagulation of milk, 421
Heat, effect on milk dialysates, 727
Homogenized milk,
effect on haircoat of calves, 41
frozen, 699
testing, 845
Hydrogen peroxide,
effect on tallowy flavor, 293

ICE cream mix,
powdered, 486, 488
spray dried, 371, 493
Inter-breed program, 480
Iodine,
effect on bulls, 555
Iodinated casein,
effect of feeding, 532, 533, 534

JUDGING dairy products, 415, 500

KETOSIS,
adrenal relationships, 523
blood composition in, 183
spontaneous recovery, 151
treatment, 131

LACTATION,
effect of stilbestrol on, 524
Lactic acid,
in dairy products, 33
Lespedeza hay,
digestibility of, 631

MALE hormone,
elimination by cows, 525
Manganese, 538
Mares milk, 163
Methionine in ketosis treatment, 131
Milk,
ammonia determination, 645
B vitamins in, 783
carmel flavored, 607
deareation of, 359
dried,
brown discoloration, 488
fluorescence, 491
keeping quality of, 486, 488, 489
peroxide value, 1
solubility, 485, 490, 492
drying, 485, 492
evaporated, 625
storage life of, 499
flavor,
effect of route delivery on, 273
tallowy, 293
formic acid in, 27
heat coagulation of, 421
homogenized, 41, 699, 845
judging, 415, 500
oxygen content, 359
phosphatase test, 507, 737
production, 345
total solids estimation, 87
Milk fever, 617, 767
Milking machines, 45, 849
Milking technique, 45
Moisture in dairy products, 639
Mold,
inhibitors, 173
Mung beans,
as protein supplement, 537

NITROGEN,
packing of dried milk, 207
retention from casein and lactalbumin, 717
Non protein nitrogen utilization, 535
Nutrient losses from hay and silage, 239

OXIDIZED flavor, 359
Oxygen,
  content of dry milk, 207
  content of milk, 359

PASTEURIZED milk,
  bacteria counts, 506
  coliform bacteria in, 651
Pastures,
  yield determination, 393, 540
Penicillin,
  in mastitis treatment, 529
Peroxides,
  in milk powder, 1
Phosphatase test,
  application to cheese, 507, 737
  application to fluid milk, 737
Phosphorus supplements, 317
Photolysis of riboflavin, 508
Powdered cream mix, 511
Pregnancy,
  effect on body weight, 657
Propionic acid and propionates,
  as mold inhibitors, 173
Protein supplements, 536, 537
Protein utilization, 717
Pulse rate of cows, 465

QUALITY,
  cheese, 157
  dried milk, 1, 55, 145, 485, 486, 488, 489, 490, 491, 492
  milk,
    effect of route delivery on, 273
  programs, 485, 495
  spray dried ice cream mix, 371, 493

RECORDS,
  use of, 477
Reproduction,
  effect of stilbestrol on, 524
Respiration rate of cows, 465
Riboflavin,
  photolysis, 508
Rock phosphate,
  defluorinated, as feed, 371
Roughages,
  alfalfa hay, 546, 551
  barn dried, 547, 549, 550
  harvesting, 547
  nutrients in, 549, 631
  palatability, 546
  silage, 239, 541, 542
Rumen,
  microflora, 542
  physiology, 543
Ruminant,
  intravenous feeding of, 526

SALT,
  method of determination, 431
Sanitation,
  milking machine, 849
Semen,
  coloring for identification, 554, 815
  dilution, 695
  preservation, 407
Shark meal,
  as protein supplement, 536, 801
Silages,
  from damaged corn, 541
  lactation factor in, 542
  nutrient losses from, 239
Stability of cream style foods, 221
Stability of frozen cream, 213
Stilbestrol,
  effect on lactation, 524
  effect on reproduction, 524
Sucrose,
  acid inversion of, 101
Sulfa drugs,
  effect on spermatozoa, 285
  in mastitis treatment, 529
Surface active agents, 751

TALLOWY flavor, 293
Testing dairy cattle,
  D.H.I.A., 473, 474, 477
  official, 480
Thyroid secretion rate, 531
Thyroprotein,
  effect on milk composition, 533
  physiological effects, 532, 533, 534
Training dairy workers, 513

UDDER,
  removal of milk from, 45
Urea,
  utilization by cows, A629
  utilization by heifers, 535

- VITAMINS,
  - A,
    - and reproductive performance, 522, 669
    - content of butter, 91
    - content of cheese, 157, 789
    - content of colostrum, 13, 515, 517, 520, 521, 809
    - fetal storage, 516
    - in blood of calves, 13, 515, 517, 519
    - placental transmission, 516, 707
  - B complex,
    - in milk, 783
    - photolysis of riboflavin, 273, 508
  - Biotin in milk, 783
  - C, (ascorbic acid)
    - effect of route delivery on, 273
    - stability in evaporated milk, 625
  - Carotene,
    - content of cheese, 13, 789
    - potency of butter, 91
    - utilization by calf, 381
    - content of mare's milk, 163
  - D,
    - effect on blood picture of cows, 514
    - role in milk fever, 617, 767
  - Niacin in milk, 783
  - Pantothenic acid in milk, 783
  - Riboflavin,
    - photolysis, 273, 508
  - supplementary for calves, 231, 517, 761

- WEIGHT estimation, 141, 544
- Washing dairy equipment, 453
- Water supplies, 510
- Weather,
  - effect on grazing, 199
- Weight estimation, 141, 544
- Weight vs. metabolic body size, 259
- Whey,
  - proteins, 503

- YEAST,
  - from cream and butter, 507

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

1. **Bioenergetics and Growth.** SAMUEL BRODY, Univ. of Missouri. 1023 pages. Illustrated. Reinhold Publishing Co., 330 W. 42nd St., New York. Price, $8.50.

In this book Dr. Samuel Brody in his characteristic thorough manner has integrated the results obtained from the researches sponsored by the Herman Frasch Foundation for Research in Agricultural Chemistry at the Missouri Agricultural Experiment Station. The work deals largely with the energetic efficiencies of animal production processes such as the production of milk, meat, eggs and work.

Space does not permit an adequate review of the contents which range over a wide field and include analytical appraisals of many livestock production processes. The literature is generously used and a page by page bibliography provided. A wealth of graphic and tabular data accompanies each chapter.

The book will be a most useful addition to the library of the research worker in the animal science field. The fact that it deals largely with specifics rather than broad generalities makes it particularly useful to the investigator. T.S.S.

2. **Dairy Bacteriology and Public Health.** C. S. BRYAN. Burgess Publishing Company, 426 S. Sixth Street, Minneapolis 15, Minn. 1945. 33 pp. $1.00.

This publication is useful as a handbook for veterinarians, milk sanitarians and teachers of microbiology as related to the dairy industry and its public health aspects.

The subject matter is divided into 12 divisions and is presented in outline form. It is assumed that teachers, students and sanitarians would have to rely upon other sources for complete information on detailed bacteriological and chemical techniques.

The material is presented in logical sequence, starting with the composition of milk and commercial milk products, grades of milk, sources and prevention of high bacterial counts in milk on the farm and in the processing plant. This is followed by the use and evaluation of different bacteriological quality tests. Essential steps are also listed for the examination and handling of water supplies to determine its potability. Dairy cleaners and cleaning of dairy equipment are discussed, emphasizing their effectiveness as sanitization agents and cleansing ability. The essential properties of cleaners are listed, such as wetting, emulsification, deflocculation, dissolving power, buffer action and water-softening ability. The final discussion deals with a suggested procedure for inspection of milk on the farm and in the plant. H.H.W.

BUTTER

3. **Vitamin A Potency of Idaho Butter.** D. R. Theophilus, Olof E. Stamberg, D. W. Bolin, and H. C. Hansen. Idaho Agr. Expt. Sta. Cir. 102. 1945.

Samples of butter manufactured in Idaho were collected semimonthly from four areas for one year. The vitamin A and carotene contents were determined by chemical procedures and the sum of the two was the vitamin A potency.

It was found that the vitamin A potency was lowest, about 12,500 I.U., in February and March, and it was highest, about 19,000 I.U., in July to October. The decrease in carotene in the winter months was greater than the decrease in vitamin A. In July the vitamin A content of the butter was 1.3 times that of March butter and the carotene content in July was 2.2 times greater than March butter. If a uniformly high level of vitamin A potency is to be maintained, it would be necessary to improve winter feeding.

A.C.D.

CHEESE

4. **Studies on the Riboflavin Content of Cheese.** I. L. Hathaway and H. P. Davis. Univ. of Nebr. Agr. Expt. Sta. Res. Bul. 137. 1945.

The riboflavin content of 27 kinds of cheeses and cheese spreads were determined by the rat assay technique. Most of the cheeses were purchased on the local market and a few were manufactured in the Nebraska Experiment Station creamery.

It was found that most of the cheeses contained from 3 to 6 micrograms of riboflavin per gram of cheese. Only cream cheese contained smaller amounts of this vitamin. Larger amounts were present in goat cheese, Mel-O-Pure, Leiderkranz, and Velveeta. Since duplicate cheeses were obtained for only two varieties, the results may not necessarily indicate differences between varieties. There appeared to be no significant difference in riboflavin content on the basis of classification of the cheeses as hard, semihard, soft, and process. A.C.D.

5. **Matting of Cottage Cheese.** Thos.. B. Harrison and W. M. Roberts, Agr. Expt. Sta., Univ. of Tenn., Knoxville. Southern Dairy Products Jour., *38*, 3: 54. September, 1945.

When cottage cheese curd mats and becomes tough and rubbery, it cannot be marketed. The Dairy Department of the University of Tennessee has made a preliminary study of the following possible factors: degree of acidity, amount of rennin, addition of fat, addition of steapsin, and enzylac. Further study under commercial conditions is being planned. F.W.B.

6. **The New York State Cheese Improvement Program.** J. C. MARQUARDT, N. Y. State Dept. of Agr. & Mkts., Geneva, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 9–16. 1944.

In 1942 and 1943 more than three-fourths of the Cheddar cheese made in New York State was manufactured from milk diverted from fluid markets. There has been some trouble due to slow acid development which has been largely overcome. About one-fourth of all the Cheddar cheese was made in five plants. About 70 per cent of the Italian cheese was made in two plants.

The program to improve cheese includes sediment testing, milk cooling, mastitis eradication, methylene blue and resazurin tests, proper can washing, improved lactic cultures, and better manufacturing procedures.

The Cheddar cheese is made chiefly from raw milk and "although cheese has never been a public health hazard, changing food habits may make it one." A.C.D.

CHEMISTRY

7. **The Component Acids of Milk Fats of the Goat, Ewe, and Mare.** T. P. HILDITCH, Dept. of Indus. Chem., Univ. of Liverpool, AND H. JASPERSON, Res. Dept., Messrs. J. Bibby and Sons, Ltd., London. The Biochem. Jour., *38:* 443–447. 1945.

Fats obtained from samples of goat, ewe and mare milks were studied by fractionation analyses of the methyl esters.

"The general similarity between goat- and ewe-milk fats has been confirmed, and differences have been indicated by the relatively higher content of decanoic and a relatively lower content of stearic acid in goat-milk fat than in ewe-milk fat.

Mare-milk fat, which has not been analyzed in detail hereto, has been shown to comprise the following acids (% by mol.); butyric 1.1, hexanoic 1.9, octanoic 4.4, decanoic 7.9, lauric 6.8, myristic 7.4, palmitic 15.4, stearic 2.4, as arachidic 0.2, decenoic 1.3, dodecenoic 1.2, tetradecenoic 1.9, hexadecenoic 7.2, oleic 16.3, octadecadienoic 6.6, linolenic 14.0, unsaturated C_{20-22} 4.0. The most arresting features are the low butyric and hexanoic acid contents and the presence of substantial proportions of linolenic acid amongst the C_{18} unsaturated acids." A.O.C.

8. **Human-milk Fat. 2. Component Glycerides.** T. P. HILDITCH AND M. L. MEARA, Dept. of Industrial Chemistry, Univ. of Liverpool.

The Biochem. Jour., *38:* 437–442. 1945. (See also previous work by same authors. Abstract 392. December, 1944.)

The method employed was to crystallize the human-milk fat systematically from acetone at various temperatures, five groups in all, and to study each group by the ester fractionation procedure.

"1. Human-milk fat glycerides consist of about 24% of di-unsaturated glycerides in which the mono-saturated group is myristic, lauric or decanoic acid, about 19% of palmitic di-unsaturated glycerides, about 20% of glycerides containing one radical each of palmitic acid, unsaturated acid, and one of the C_{10}, C_{12}, or C_{14} acids, about 14% of mono-unsaturated-palmitosterins, and minor proportions of various other mixed glycerides.

"2. The proportions of mono-palmito-glycerides and of glycerides containing one radical of either of the lower saturated acids have close resemblances to the proportions of the corresponding groups of glycerides in cow-milk fats, and thus suggest that the origin of the short-chain glycerides may be the same in both fats.

"3. It is probable that the di- and tri-unsaturated glycerides of human-milk fat contain one radical of linoleic or other unsaturated acid, the remainder being oleic, and that the mono-unsaturated glycerides are almost wholly mono-oleo-glycerides. It is also possible that the source of milk-fat short-chain glycerides may be linoleic-glycerides rather than oleo-glycerides as previously suggested." A.O.C.

9. **Estimation of Sucrose and Lactose in Binary Mixtures with Particular Application to Sweetened Condensed Milk.** H. H. Browne, Div. Dairy Res. Lab., Bur. Dairy Indus., U. S. Dept. of Agr., Wash., D. C. Jour. Indus. Engin. Chem., Analyt. Ed., *17,* No. 10: 623. Oct., 1945.

This method is based on the fact that sodium bi-sulfite decreases the optical rotation of aldose sugars and that the optical rotation of a mixture of two sugars, at least one of which is such a sugar, can be made a linear function of the concentration of each sugar. The method consists of two steps: the determination of the percentage of total sugars by means of the refractometer and the measurement of the optical rotation of a solution containing a known percentage of total sugars in the presence of sodium bisulfite. Data are presented for the optical rotation of sucrose-lactose mixtures and for the application of the method to a sample of sweetened condensed milk. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

10. **Continuous Process Developed for Making Lactic Acid from Whey.** Anonymous. South. Dairy Prod. Jour., *38,* 3: 68. Sept., 1945.

Lactic acid, a chemical suitable for many industrial uses and one that can be made by fermenting almost any kind of sugar, was first identified in sour milk about 160 years ago. But it was never made commercially from milk sugar until after the Bureau of Dairy Industry process was developed, although it had been made in this country from other sugars for more than 50 years.

The fermentation in whey may be carried out continuously by the use of a mixed culture of a lactobacillus and a mycoderm (a yeast-like organism), the acidity of the whey being kept within a certain range by the addition of slaked lime. Over 95 per cent of the milk sugar was thus converted to lactic acid.

A hundred pounds of whey contains five pounds of milk sugar. Estimating the value of sugar at 2½ cents a pound, whey would be worth 12½ cents per hundred pounds. Lactic acid has many uses itself and is also the material from which other chemicals can be made. F.W.B.

11. Consumer Acceptance of Dried Milk in Houston, Texas. ANONYMOUS. South. Dairy Prod. Jour., *38*, 3: 66. Sept., 1945.

Under wartime pressures the production of dried milk has been expanded. Consequently, when the wartime needs come to an end, there will be the problem of whether or not dried milk will be accepted by the general public. In many sections of the country, especially in the Southern deficit milk-producing areas, it is possible that consumption of milk could be increased by merchandising dried milk of a good quality.

At the request of the Division of Marketing and Transportation Research, the Division of Program Surveys of the U. S. Department of Agriculture conducted a survey to determine consumer's reaction to dried milk in an area which has experienced a milk shortage. Houston, Texas, represents such an area. For more than a year, dried whole and dried skim milk have been sold in Houston. Two hundred fifty-three buyers of dried milk and 479 non-buyers were interviewed.

Dried milk has been purchased in approximately one-sixth of the households in Houston. Many more people have bought dried whole milk than have bought dried skim milk. Most people who have not bought dried milk did not know that it is being sold. Dried milk has been bought because of a scarcity of fresh milk, simply as an experiment or because it is economical. Nearly one-half of the purchasers have bought only one package.

People think that the best use of dried milk is for cooking or baking. Approximately 90 per cent of the people who have used dried milk used it in reconstituted form. About one in five of the users say its preparation is difficult. One-half of the people who have bought dried milk say they expect to use it after the war. Dried whole milk will be in greater demand than dried skim milk. F.W.B.

FEEDS AND FEEDING

12. The Utilization of Urea in the Bovine Rumen. 4. The Isolation of the Synthesized Material and the Correlation Between Protein Synthesis and Microbial Activities. J. A. B. Smith, Hannah Dairy Res. Inst., Kirkhill, Ayr, The County Technical Col., Guildford. The Biochem. Jour., *38:* 496–505. 1945.

"Rumen liquor has been incubated *in vitro* with and without added carbohydrates. The synthesis which results under favourable conditions has been estimated by isolating the synthesized material as an amorphous powder which consists mainly of bacteria.

"Synthesis of protein is accompanied by synthesis of a starch-like polysaccharide and by an increase in the iodophile population of the medium.

"The greater portion of the synthesis is attributed to the mass of micro-iodophile bacteria which abound in the rumen. Protozoa do not appear to contribute to the synthesis.

"Typical analytical figures for the sediment containing the synthesized material are 0.5% moisture, 36.3% protein, 46.6% polysaccharide, 9.5% lipoid matter and 6.2% ash. These values are very similar to the corresponding values for a typical feeding stuff such as linseed cake." A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

13. Milk Abounds in Anti-Anemia Factors. Anonymous. South. Dairy Prod. Jour., *38,* 3: 28. Sept., 1945.

Anemias originate either from the loss or destruction of red blood cells and/or hemoglobin or from a failure in their formation. The nutrients which apparently help to build and maintain red blood cells and hemoglobin are protein, iron, copper, riboflavin, pyridoxine, niacin, and pantothenic acid. Experiments on human beings lead to the conclusion that a diet adequate in all respects should be recommended for the prevention of nutritional anemias. All the nutrients known to be involved in the prevention and control of anemia occur in milk in appreciable quantities with the exception of iron and copper which are present in small amounts. F.W.B.

HERD MANAGEMENT

14. Saving Labor for the Dairy Farmer. John A. Hitchcock, Vt. Agr. Expt. Sta. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 47–52. 1944.

During a period of four months various alterations and adjustments were made on the dairy farm of Leonard Clark of Fletcher, Vt., to affect labor economies in the dairy barn. These changes may be grouped under (1) rearrangements within the barn, (2) improved equipment, (3) improved work routines, especially timed milking, and (4) improved positioning of equipment and supplies.

"In terms of hours and minutes the reduction in time was from 5 hours, 44 minutes, to 3 hours, 39 minutes, a saving of 2 hours, 5 minutes; in terms of miles that in travel was from 3¼ to 1¼ miles, a saving of 2 miles. These savings represent 36 and 62 per cent, respectively, of the original time and travel. Two hours a day is equivalent to more than 60 12-hour days a year, a good full 2 months work; 2 miles a day is equivalent to 730 miles a year."

J.G.A.

ICE CREAM

15. **The Use of Stabilizers in Ice Cream.** H. A. Bendixen, Wash. Agr. Expt. Sta., Pullman, Wash. South. Dairy Prod. Jour., *38,* 1: 68. July, 1945.

Mixes and ice creams containing 0.5 per cent oatgum were compared with similar products containing 0.22 per cent sodium alginate. The oatgum was mixed with four times its weight of sugar and sprinkled in the mix at about 80° F. The sodium alginate was dispersed in cold water and added at 160° F. The mixes contained 11–12 per cent fat and 34–35 per cent total solids.

When no egg yolk powder was used the use of oatgum produced a slightly higher viscosity than the use of sodium alginate. The oatgum mixes increased 20–30 per cent in viscosity during aging for three days at 38–40° F. while the viscosity of the sodium alginate mixes increased very little.

When 0.4 per cent egg yolk powder was used in the mixes the oatgum mixes usually had only slightly higher viscosity. The whipping ability of the oatgum mixes was very similar to that of the sodium alginate mixes. There was no difficulty in any case, in obtaining 100 per cent overrun, with or without egg yolk powder. The oatgum mixes showed a higher melting resistance at 75–80° F. room temperature than the sodium alginate mixes. The slightly greater residue left by the oatgum mixes on a wire screen after melting was not significant.

From the standpoint of flavor, texture and general consumer acceptance, there was little practical difference between the ice creams containing oatgum and those stabilized with sodium alginate. F.W.B.

16. **Shrinkage Defect in Ice Cream. Part II.** Denzel J. Hankinson and Chester D. Dahle. South. Dairy Prod. Jour., *36,* 5: 34, 36. Nov., 1944.

Variations in fat content from 10 to 16 per cent caused little if any effect on shrinkage, when the sugar content of the non-fat portion was kept constant. Ice cream from a mix containing 10.5 per cent serum solids showed slightly more shrinkage under vacuum treatment than that from a mix containing 8.0 per cent serum solids when the other mix constituents were constant. No shrinkage occurred in the latter samples without vacuum treatment. More shrinkage was evident in ice cream containing plain condensed skim milk than in that containing superheated condensed skim milk under all the test conditions used in these experiments.

Developed acidities of 0.015, 0.025 and 0.040 per cent had little if any effect upon shrinkage. Ice cream containing cane sugar or cane sugar with a substitution of 30 per cent of corn syrup, Sweetose, Cerelose, Frodex and honey on a dry basis in different lots showed no appreciable shrinkage without vacuum treatment. Vacuum-induced shrinkage was least in the ice cream containing corn syrup followed by those containing Sweetose, Cerelose and Frodex which showed about the same shrinkage. The ice cream containing honey showed the most shrinkage.

The use of 0.3 per cent gelatin and no stabilizer in separate mixes resulted in the least vacuum-induced shrinkage while 0.4 per cent Vesterine, 0.0225 per cent Articol, and 0.25 per cent Dariloid resulted in slightly more shrinkage. No appreciable change occurred without vacuum treatment.

A forewarning temperature of 175° F. in the skim milk before condensing resulted in slightly less vacuum-induced shrinkage than temperature of 125° F., 150° F., or 200° F. All samples not vacuum-treated showed no significant changes.

A mix containing butter showed less shrinkage than a similar mix containing 0.5 per cent dry egg yolk also. When egg yolk and egg whites were added to mixes containing cream there was less shrinkage than in mixes from which they were omitted. Shrinkage loss was greater in ice cream produced from vacuum-pan mixes than in cases in which the mixes were made by the conventional method.

It is impossible to conclude that either the vacuum treatment or an external pressure treatment is a satisfactory method for studying the effect. Either treatment may permanently destroy many of the individual air cells. Shrinkage occurs more readily at a higher overrun. F.W.B.

17. **Production Limited by Scarcity of Sugar.** C. D. Dahle, Dairy Dept., Pa. State College, State College, Pa. Ice Cream Field, *46,* No. 3: 13. 1945.

The sugar shortage forces ice cream manufacturers to get more sugar alternates or reduce the sweetness of their ice cream. It is stated that 12 per cent sugar mixes will be acceptable in place of higher sugar content mixes, but it is recommended that fairly high serum solids be used to offset the low sugar content. The author also confirms other reports showing that corn syrup and "Frodex" have greater sweetening power than was originally assigned them.

Reducing the amount of sherbet manufactured will enable the industry to make more ice cream and a greater total gallonage. Calculations are tabulated to show that if 900 pounds of sugar are used in the manufacture of ice cream, 1203 gallons can be produced; whereas if the same sugar were used to make sherbets, only 432 gallons would be produced. W.C.C.

18. **A Sound Frozen Food Business Is Built by a Mid-West Ice Cream Manufacturer.** MARJORIE SMITH. Ice Cream Field, *46,* No. 3: 42. 1945.

A brief account is given of the growth of the Squire Frozen Food Lockers and Squire Ice Cream Company of Shenandoah, Iowa. Squire believes that locker plants furnish good outlets for wholesale manufacture of ice cream and "locker traffic" increases the sale of ice cream and other dairy products for retail operation. W.C.C.

19. **Use Ascorbic Acid to Blanch Apricots and Peaches.** ANONYMOUS. Ice Cream Field, *46,* No. 3: 51. 1945.

Charles Collins, Frostcraft Packing Company, San Jose, California, is quoted as saying that a new process for apricots and peaches, which utilizes ascorbic acid (Vitamin C) in place of steam blanching, preserves the natural freshness and flavor of the fruit and prevents discoloration at the time of defrosting.

Fresh fruit was first treated on a commercial scale in 1941, but lack of availability of ascorbic acid during the war period has prevented its general use until now.

Various research agencies have cooperated in the project according to the report, and it is stated that over a million pounds of apricots were packed by this process this year. W.C.C.

20. **New Orleans Claims First American Ice Cream.** ANONYMOUS. Ice Cream Field, *46,* No. 3: 26. 1945.

A clipping from *Times-Picayune,* a New Orleans newspaper, declares that ice cream was first served in New Orleans in 1808—four years before President Madison took office. This discredits the belief, held by some, that Dolly Madison introduced ice cream to America by serving it at the White House during the administration of President James Madison. W.C.C.

21. **Egg Solids Improve Ice Cream Quality.** B. I. MASUROVSKY, Res. Editor, Ice Cream Trade Jour., *41,* 9: 56. Sept., 1945.

With the production of dried eggs greatly increased during the past three years, it has been suggested that the ice cream industry might use them in good quantities in the post-war period. Experimental work has shown that 0.5 per cent egg yolk solids improves the whipping property of the ice cream mix.

Dried eggs should be kept in proper containers in a dry storage space and when a container is opened its entire contents should be used to prevent spoilage.

Dried eggs may be incorporated into the mix by mixing them with part of the sugar and adding them to the mix when it reaches 100° F. For French ice cream 1.5 per cent of dried yolks may be used.

The following information is taken from Circular No. 583 of the U. S. Dept. of Agriculture, entitled: "Eggs and Egg Products," and should serve as a guide in figuring the amount of egg yolk: "A case of 30 dozen eggs weighs about 42 to 43 pounds net and yields approximately 35 pounds of liquid whole egg. One hundred pounds of shell eggs produces commercially about 84 pounds of liquid whole egg. Twenty whole eggs, 36 whites, or 48 yolks make 1 quart of product. The liquid egg from 9 to 11 shell eggs will weigh about 1 pound, and 17 to 20 whites or 19 to 22 yolks weigh one pound. One pound of dried eggs can be made from 29 liquid eggs; 1 pound of dried yolk from 48 yolks, and 1 pound of dried albumen from 70 whites."

W.H.M.

22. Liquid Sugar's Rôle in Ice Cream Plants. B. I. MASUROVSKY, Res. Editor, Ice Cream Trade Jour., *41,* 6: 52. June, 1945.

The use of liquid sugar in ice cream has proven advantageous from a sanitary standpoint and has provided a convenient way of furnishing sugar solids for ice cream. In using liquid sugar, its water content must be taken into consideration. A 38° Baume liquid contains 3.28 pounds of water and 8 pounds of sugar solids per gallon. To make 100 gallons of ice cream mix (900 lb.) containing 14 per cent sugar solids would require 900 times 0.14 or 126 lb. of dry sugar and 126 divided by 8 or 15.75 gallons of liquid sugar. The amount of liquid sugar would contain 15.75 × 3.28 or 51.6 pounds of water which must be taken into consideration in calculating the remaining ingredients of the mix. Considerable time and labor can be saved by using liquid sugar.

The general picture of handling dry sugar is as follows: 1. Unloading of bags from delivery trucks. 2. Lifting of bags from elevators and carts into stock rooms or storage place. 3. Carting the bags to the processing room. 4. Cutting open the bags and dumping the sugar into the tanks. 5. Handling of empty bags.

In contrast, the general picture of utilizing liquid sugar in an ice cream plant is resolved into two steps: 1. Pumping the liquid sugar from the tank truck to storage tanks. 2. Metering the required amounts of liquid sugar by the turn of a valve, from storage tanks into the mixing receptacles or pasteurizing tanks. Furthermore, the liquid sugar system provides a more sanitary handling of sugar solids by eliminating possible contamination through dust, dirt, bag lint and other foreign matter.

Already a large number of ice cream manufacturers have accepted the closed system of handling liquid sugar, because in a progressive food industry steps must be taken to improve the finished product by eliminating contamination. W.H.M.

23. **Dehydrated Sweet Potato Flour in Ice Cream.** A. J. Gelpi and P. G. Kennedy, La. State Univ., Baton Rouge, La. South. Dairy Prod. Jour., *38,* 3: 38. Sept., 1945.

Recently dehydrating plants have been constructed which are manufacturing millions of pounds of wholesome and nutritious dehydrated sweet potatoes low in starch content and high in Vitamin A and carotene content. This product has been used satisfactorily in ice cream, sherbets, and ices at Louisiana State University.

The amount of sweet potato flour which should be added to a mix as a stabilizer depends upon the composition of the mix as well as the condition of processing. The range between 0.75% and 1% will usually be found satisfactory in most instances.

Ice cream mix stabilized with dehydrated sweet potato flour was found to possess the following properties: 1, Slightly more color than gelatin mixes; 2, A uniform viscosity during aging. The viscosity of the freshly made mix was practically the same as the aged mix; 3, A maximum whipping ability immediately after being viscolized and cooled to 40° F.; 4, No tendency toward wheying-off.

The ice cream made with mix stabilized with sweet potato flour was found to possess the following properties: 1, A color slightly darker than gelatin ice cream; 2, Body and texture comparable to gelatin ice cream; 3, Good water binding capacity; 4, No tendency toward shrinking; 5, Smooth, clean and satisfactory melt-down; 6, It was also observed that sweet potato stabilized ice cream withstood head shocking to a greater extent than ice cream containing other stabilizers.

In experimental trials it was found that sweet potato flour could replace as much as 3% of the serum solids in ice cream. The sweet potato flour not only replaces 3% of the serum solids but at the same time replaces the necessity of additional stabilizer, 1% of the sugar content and practically all the color. The finished product has good flavor, excellent body and texture, and excellent whipping qualities as 130% overrun was reached in five minutes after ammonia was shut off.

It should be understood that the use of sweet potato flour or any other product in ice cream as a substitute for part of the non-fat solids is to be recommended only during periods of emergency such as have existed in the immediate past. F.W.B.

24. Educating Fountain Clerks. G. P. GUNDLACH AND C. W. ESMOND. Ice Cream Trade Jour., *41,* 9: 70. Sept., 1945.

During the war many ice cream sales departments have maintained their sales department somewhere near full strength. Now that the war is over, sales managers will be faced with the problem of training new sales people. Providing manuals, making demonstrations and showing pictures will help, but the best way is for the sales managers of the wholesale manufacturers to personally teach and demonstrate and counsel and admonish and inspire and everlastingly "follow through" with the individual clerks serving in the stores of their dealers. W.H.M.

25. Fourteen Points for Post War Plant Operation. H. A. ACKERMAN, Dairy Mfg. and Res. Bur., G. P. Gundlach & Co., Cincinnati, O. Ice Cream Trade Jour., *41,* No. 8: 39. Aug., 1945.

Ice cream manufacturers should put good operating practices into effect now, if they expect to meet post war competition successfully. Some of the factors which are characteristic of sound plant operation are: 1, Good personnel relations; 2, Well-planned buildings and plant layouts; 3, Well-planned plant operations; 4, Large hardening rooms (at least large enough to hold 5 days maximum production); 5, Preventive maintenance; 6, Conveyors to handle material; 7, Good supervision; 8, Line production methods; 9, Improved cleaning and sterilizing operations; 10, Good house-keeping methods; 11, Quality control by means of a good laboratory; 12, Close attention to overrun control; 13, Proper plant accounting; 14, The use of good fruits and flavors. W.H.M.

26. Ice Cream with Reduced Sugar Content. HARRY PYENSON AND P. H. TRACY, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Ice Cream Trade Jour., *41,* 9: 30. Sept., 1945.

During the fourth quarter of 1945 ice cream manufacturers are limited to 50 per cent of the sugar used during the base period. With unlimited quantities of butter fat and milk solids now available, manufacturers are trying to stretch their supply of sugar as far as possible.

Experiments have been conducted at the University of Illinois to study the effect of reduced amounts of sugar on the body, texture and flavor of ice cream. The results of these experiments showed that a reduction in sugar content, (1) raises the freezing temperature of the mix, (2) raises the drawing temperature, (3) speeds up whipping of the mix, and (4) raises the dipping temperature.

The sugar content should not be lowered to less than 10 per cent in plain ice cream and an improvement in body texture of low sugar ice cream can be effected by raising the solids-not-fat up to 13 per cent. Cereal solids may also be used for this purpose. In making chocolate ice cream with a reduced sugar content, it is advisable to lower the amount of chocolate flavor used. W.H.M.

27. **Three-year Analysis of Wartime Sales.** VINCENT M. RABUFFO, Editor. Ice Cream Trade Jour., *41,* 9: 38. Sept., 1945.

A report released by the International Association of Ice Cream Manufacturers shows that 1944 sales of all products increased 9.09 per cent in 1944 over 1943. The sales in 1944 were divided as follows: Ice cream sales to civilians, 67.21%; ice cream sales to armed forces, 13.85%; ice milk and low fat products, 2.33%; milk sherbets, 10.58%; fruit ices, 6.03%. The sale of sherbets increased from 2.95 per cent of the total sales in 1942 to 11.98 per cent in 1944, and fruit ices from 2.32 per cent in 1942 to 6.83 per cent in 1944. Sales in Canada increased 3.51 per cent in 1944 over 1943. W.H.M.

28. **Preparation of Irish Moss Extracts for Use as a Jelling and Stabilizing Agent in Foods.** C. O. FULTON AND MISS B. METCALFE, Nat. Res. Council Lab., Ottawa, Canada. Canad. Jour. Res., *23,* Sec. F, No. 4: 273. 1945.

Hot water extracts of bleached Irish moss were clarified by treatment with activated charcoal and filtration through diatomaceous earth after which the extract was concentrated by freezing and removing the ice. Further drying to a moisture content of 8 to 10 per cent was accomplished by means of a fan after which the product was ground in a Wiley mill.

The material was effective in stabilizing chocolate milk in the same concentration as a product now on the market. It was preferred to agar-agar in jellied canned chicken but was not a complete substitute for pectin in grape jelly. O.R.I.

29. **Use of Dried Eggs in Ice Cream.** CARL FRISCHKNECHT, Senior Marketing Specialist, War Food Administration, Wash., D. C. South. Dairy Prod. Jour., *37,* 6: 34, 48. June, 1945.

Needs of the war period have resulted in 121 egg-drying plants in this country in 1944 compared to only 16 in 1941 and a production of 320,742,000 pounds of dried eggs in 1944 compared to 7, 487,000 pounds in 1940. Serious thought should be given now to practical uses of dried egg products during the post war period.

It is apparent from the information presented that dried egg yolk and dried whole eggs of high quality have a definite place in the manufacture of all kinds of ice cream. They are less expensive per unit and they can be stored and added to the mix with less waste and greater ease than fresh or frozen eggs. It is quite generally agreed that the addition of dried eggs improves the whipping qualities of the mix, reduces the time required to obtain the overrun, adds color; increases the food value, smoothness and stiffness; and makes the ice cream more resistant to melting. Mixes containing one per cent and two per cent of egg solids each received good consumer acceptance in taste test panels conducted recently. Further research work and educational programs are needed. F.W.B.

MILK

30. Cost of Producing Milk in Illinois Portion of the St. Louis Milkshed.

R. H. Wilcox and C. S. Rhode. Ill. Agr. Expt. Sta. Bul. 515. 21 pages. Aug., 1945.

Cows averaged 7,762 pounds of milk yearly in the 198 herds included in this study. Feed expenses constituted 44.5 per cent and man labor 25.5 per cent of the net cost of producing milk. The remainder, 30 per cent of the cost, was made up by thirteen other items. The higher producing cows required less feed and labor per unit of milk produced than the lower producing cows. Hay and pasture produced the greatest feed variation. The facts concerning feed and labor costs are used as the basis for working out formulas and graphs. These figures compute milk costs for herds producing from 5,500 to 9,000 pounds per cow yearly. The only variable in the formulas are the local price of shelled corn and the cash wages of the hired help.

Some of the causes for variations in cost are given. The unit cost of production is reduced by (1) high production per cow, (2) large herds, (3) milking machines. The average yearly profit per cow in these herds studied in 1938 and 1939 was $11.19. The herds in which high seasonal variations in milk production were kept down were more profitable than those in which production was allowed to vary widely. -Seasonality of production was influenced mainly by the time of year the cows freshened and the feed program followed. H.P.

31. Coliform Bacteria in Pasteurized Milk—the Utilization of Laboratory Control Methods.

Robert S. Breed, N. Y. State Agr. Expt. Sta., Geneva, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 157–167. 1944.

Growth of coliform bacteria in raw milk makes the coliform test of little value in indicating initial mammal pollution. Furthermore, the coliform bacteria may not come from mammal contamination.

The situation is different for freshly pasteurized milk. McCrady has shown that freshly pasteurized milk in 1 ml. quantities can be free from coliform bacteria. The coliform test is widely used on pasteurized milk to indicate recontamination after pasteurization. It is doubtful if tests on pasteurized milk 24 hours old has much value due to the possibility of growth at unknown temperatures.

Two agars and two fermentation tube technics are given in Standard Methods. The author favors formate ricinoleate broth but notes that agars are used as extensively as broths. While certain bacteria other than coliform gave positive broth tests, these organisms are not common in milk and most of them (except some spore-formers) are destroyed by pasteurization.

A.C.D.

32. Coliform Bacteria in Pasteurized Milk—as Affected by Using an Acid Detergent for Can Washing. Henry W. Lehmkuhl, Milk Plant Specialties Corp., Rochester, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 149–156. 1944.

Milk cans have generally been considered to be a source of alkaline-reacting, proteolytic types of bacteria rather than coliform.

Gluconic acid is common in acid washing solutions, the proprietary liquid contains the equivalent of about 25% gluconic acid and 2½% is recommended. The actual concentration of acid in the spray is about 0.6%. The undiluted liquid has a pH of 2.1; a 1% solution has a pH of 2.9 in distilled water and 3.1 in Rochester tap water. It was found that gluconic acid could not be used by coliform bacteria as a source of energy. A 2½% solution destroyed them in 5 seconds.

One milk plant was using two washers for milk cans, one with alkali and the other with acid washing solutions. The alkaline solution was maintained at 0.05% alkalinity and the acid solution was also alkaline, pH 8.3. Both washers did good work and cans were free from coliform bacteria.

Another plant using acid washing solution for shipping cans failed to produce physically clean cans. The cans were not prewashed but were rinsed, acid-sprayed, acid-washed at 160° F., hot-water sterilized, and hot-air dried. Counts were 150,000,000 to 50,000,000,000 per five-gallon can and five out of six cans were coliform-positive. A.C.D.

33. Coliform Bacteria in Pasteurized Milk—Seasonal Fluctuations. C. W. Weber, N. Y. State Dept. of Health, Albany, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 139–148. 1944.

Over 10,000 coliform tests were made in three health departments during three years. Positive tests showed gas in one or more of three fermentation tubes each inoculated with 1 ml. of pasteurized milk.

The results on pasteurized milk, cream, chocolate milk, cultured milk, and frozen desserts all show the lowest percentage of positive milks in cold weather and the greatest number in midsummer. The averages for percentages of samples coliform-positive showed a direct correlation to the mean average temperature of New York State. There was no correlation between the percentages of the samples with standard plate counts over 30,000 and the mean average temperature. "The increase in positive coliform tests in the warm months is due to growth of the organisms in the milk and to a greater degree of contamination of the milk, the latter probably being the dominant factor." A.C.D.

34. Coliform Bacteria in Pasteurized Milk—Unsuspected Sources. J. F. JANSEN, Sheffield Farms Co., Oneonta, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 135–138. 1944.

Inspections were made of two bottling plants having trouble with coliform bacteria. The use of new and inefficient help was the principal problem. Instances of various disregards for sanitary practices were found. One boy drank from the milk bottles. Another lad rinsed sterilized cans with cold water from a hose to cool them before filling with pasteurized cream. Some valves were neglected. On a double holiday 16 out of 35 employees did not show up for work for three days. Some equipment was not clean. It was decided under such circumstances that until efficient help could be secured again chlorine sterilization would be eliminated. Sterilization by passing steam through the equipment for one-half to one hour greatly reduced coliform-positive samples of milk. Steam must not be used on a cabinet cooler refrigerated by brine. A.C.D.

35. Coliform Bacteria in Pasteurized Milk—Public Health Significance. J. M. SHERMAN, Cornell Univ., Ithaca, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 125–134. 1944.

The presence of coliform bacteria in milk represents direct or indirect contamination with undesirable (though not necessarily dangerous) materials. They cause undesirable fermentations.

Coliform bacteria were once thought to be exclusively of fecal origin. Rogers and Clark classified them into two groups on the basis of ratio on carbon dioxide to hydrogen in the gas evolved. The one group produced equal amounts of gas and were in the coli group; the other group produced 1.5 to 3 times as much carbon dioxide as hydrogen and were in the aerogenes group. Then Rogers, Clark, and Evans found the aerogenes group were found chiefly on grains and vegetation. Clark and Lubs developed the simple methyl red test to differentiate the group. Levine differentiated the two groups by the Voges-Proskauer test for acetylmethylcarbinol. Finally, Ayers and Clemmer showed coliform bacteria grew in partially cooled milk so that in such milk there is no correlation between numbers and initial contamination.

The presence of coliform bacteria in pasteurized milk indicates after-pasteurization contamination. A.C.D.

36. Structural versus Operational Inspections. M. ALLEN POND, U. S. Public Health Service, Washington, D. C. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 109–124. 1944.

In 1940, a study was made of the effectiveness of inspection service for an eastern city of 75,000 population. There were 1040 farms in a radius of 70 miles supplying 42 pasteurization plants. Milk consumption was one pint per capita per day.

Ten important structural and equipment factors considered important in sanitary milk production were found to be unrelated to the number of farm inspections. However, there was a positive relationship between plant defects and number of inspections. There was no relationship between structural improvements on farms or in plants and the numbers of inspections. Most inspections were made on their farms and plants producing milk of poorest quality. Milk quality was not related to structural quality either on farms or in plants. More farms per day were inspected when the farms were located far from the city.

It was pointed out that inspections should be made on farms and in plants on the basis of laboratory reports and during actual operation. About 20% of the farms were inspected without seeing anyone. A.C.D.

37. Present and Future Production Problems in the New York Milkshed. C. J. BLANFORD, New York Metropolitan Milk Market Area, New York, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 87–100. 1944.

In studying the significant alterations in the fluid milk industry in the New York Metropolitan milkshed during the war, it is best to consider the changes as (1) changes in consumption and (2) changes in production.

Fluid sales in the New York area in 1944 are 19% higher than in 1940. The requirements are 326,000,000 pounds a month compared with 275,000,000 pounds a month in 1940. Sales of fluid cream have been regulated by quotas and the fat content limited to 19%. To some extent increased milk sales have been offset by decreased cream sales.

There has been a diversion of milk from New York to other large eastern cities so increased consumption of some other eastern cities exceeded that of New York. In 1941, the New York milk pool sold 313,000,000 pounds into other markets and in 1943 this amount had increased to 545,000,000 pounds. Since 1940 the number of milk plants supplying New York decreased from 496 to 450. The producers delivering milk to New York City plants decreased from 60,300 to 51,500 during the same time. It is believed that of these 8,800 fewer dairy farms, slightly over half sold to the 46 plants that left the market and the balance quit producing milk. The production of farms that remained in the pool increased 12% while the farms decreased 15%. The tendency has been to produce more milk during the summer season. A.C.D.

38. Uniform Design of Milk Houses. J. D. BOHN, Sheffield Farms Co., 524 West 57th St., New York, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 73–85. 1944.

Two years ago this association appointed a special committee to develop plans for uniform designs of milk houses that would be acceptable in the New York area. This committee enlisted the cooperation of H. W. Riley, Cornell University, and held several meetings with various groups represented. As a result plans were recommended and were presented with drawings and specifications.

Editor's note: Since this address a brief circular has been published entitled "Approved Milk House Construction" which is based upon the work of this committee. It is stated that "Milk houses constructed in accordance with these plans will meet the requirements of the following agencies": State Health Departments of New York, New Jersey, Pennsylvania, Vermont, and Connecticut; Municipal Health Departments of New York City, N. Y., Newark, N. J., Jersey City, N. J., Plainfield, N. J., Westchester County, N. Y.; Rutgers University, Pennsylvania State College, and Cornell University. A.C.D.

39. Local or Centralized Milk Inspection. G. W. MOLYNEUX, Westchester County Dept. of Health, White Plains, N. Y. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 33–46. 1944.

The present Sanitary Code of New York State, exclusive of New York City, places responsibility for the sanitary control of milk supplies with the local health officers. The development and problems of this system of inspection were discussed. An attempt was made last year to place all dairy farm milk inspection under the control of the New York State Department of Health.

The bill, which was defeated, would have given central state inspection of all dairy farms. In cities with populations over 50,000 the inspection and control of milk within the respective borders of each city would be by the City Board of Health. City Boards of Health would control the inspection of pasteurization plants, distribution, permits, etc., and would even be able to reject raw milk not considered of proper quality. In cities less than 50,000 population, the entire inspection even within the city would be under state supervision. Such a central inspection would tend to give one uniform inspection per farm throughout the entire state and also for out-of-state shippers. The state would take over present city milk inspectors to do the work.

The discussion brought out that the original bill contained some economic features also and that some cities feared that less rigid standards would be enforced on the dairy farms. A.C.D.

40. Yami Yogurt—A Real Milk Food. ANONYMOUS. South. Dairy Prod. Jour., *37,* 5: 48. May, 1945.

Yogurt, a lactic cultured dairy product, has been a staple food in the middle East for many years and has been accepted with fervent zeal in Europe. The re-introduction of yogurt in the United States under the name of yami yogurt by the Yami Yogurt Products Company of Beverly Hills, California, has met with outstanding success.

The success in merchandising the product is attributed to its being offered as a staple food at a reasonable price rather than as a medicine, a forceful advertising campaign, and the use of a scientifically controlled culture. The Yami Yogurt Company and the Rosell Bacteriological Dairy Institute in Canada have developed literature, leaflets and posters for the promotion of sales. F.W.B.

41. New Method Keeps Processed Cream Fresh for Year or Longer. ANONYMOUS. South. Dairy Prod. Jour., *38,* 1: 48. July, 1945.

A new method of processing and sterilizing cream to keep cream pure, fresh and tasty for a year at room temperature is announced by Westinghouse Electric and Manufacturing Company, after six years of research by the California Milk Products Company, Gustine, California.

The processing includes the addition of 0.25 per cent vegetable "stabilizer," "flash sterilization" in four minutes at 260–280° F., bottling and hermetically sealing in a room freed electrically from dirt and bacteria by the use of the Precipitron air cleaner and ultra-violet rays produced by Sterilamps. When vital military requirements permit, other producers will be licensed to supply civilians, according to Mahlon K. Jordan, Vice-President of the Company. F.W.B.

42. Freezing Milk in Brick Form. GLORIA A. NERENBERG. Ice Cream Trade Jour., *41,* 6: 26. June, 1945.

A process has been developed at the Pennsylvania Agricultural Experiment Station by F. J. Doan and L. G. Leeder for the manufacture of frozen concentrated milk. Briefly, the process consists of pasteurizing good quality milk at 180° F. for 15 minutes, followed by condensing to one-third its original volume. The condensed product is homogenized, using 3000 to 3500 pounds pressure and then frozen in a continuous or batch ice cream freezer. After packaging the partially frozen milk, it is then placed in a freezing room at 10° F.

Frozen concentrated milk may be stored for several weeks at temperatures of $-10°$ F. and below. When reconstituted by adding two parts of hot water, the product resembles fresh fluid homogenized milk. Professor Doan predicts that this new product may be used in many places where fresh milk is not available. W.H.M.

MISCELLANEOUS

43. **Wrapping and Packaging Materials for Frozen Food Lockers.** P. O. Shuttleworth, Zellerbach Paper Co., San Francisco, Calif. Refrig. Engin., *50*, 4: 311. Oct., 1945.

It is pointed out that moisture-vapor-proof type wrapper should be used for all products that may be frozen by locker plants, but cost must be watched in order that it may not become prohibitive—a balance between good protection and cost must be struck. It is suggested that a locker operator should exercise complete supervision over all patron-wrapped packages received even to the point of repackaging. Patron education in proper wrapping of foods for freezing is indicated as a means to insure customer satisfaction with his own home-prepared products. The locker operator should encourage diversification of items in a customer's locker. It is stated that the rectangular carton has been the style of frozen food container in recent years because of its space-conserving quality. Cellophane available in several special types seems to be the most satisfactory vapor barrier wrapper for general use for protecting frozen foods because of its impervious nature and the ease with which it may be heat-sealed. The prediction is made that home freezing units will become an asset to the locker plant rather than a competing item. L.M.D.

44. **Insulation and Its Uses. Part I.** P. Edwin Thomas, Senior Mechanical Engineer, Wash., D. C. Refrig. Engin., *50*, 4: 319. Oct., 1945.

Cold storage insulating materials may be divided into two kinds: (a) thermal insulation, and (b) sound insulation; also into four classes; (1) animal, (2) mineral, (3) chemical, and (4) vegetable; and then both kinds and classes may be divided into (a) housing, (b) industrial, (c) air conditioning, (d) food processing, and (e) food preservation. The paper is devoted chiefly to the thermal insulation aspect of the kinds of materials now produced and their adaptability to the several classes of application, the author pointing out the advantages of the various materials, their limitations and the service which they best may perform. L.M.D.

45. **A Simple Method of Sealing Gas- or Vacuum-Packed Tins.** W. A. Bryce and H. Tessier, Nat. Res. Council Lab., Ottawa, Canada. Canad. Jour. Res., *23*, Sec. F, No. 5: 304. 1945.

A method of soldering tins within a desiccator or other chamber is illustrated and described. A $\frac{1}{16}$-inch hole in the center of the can cover is sealed with a pellet of solder, the heat for which is furnished from an electric heating coil of No. 18 chromel wire. The coils are clamped into position before placing the cans in the desiccator. When ready to seal the heating circuit is closed. The pellet of solder, previously hung in the upper part of the coil, is melted and drops on the area about the hole, producing an effective seal. O.R.I.

46. Reduction of Spatial Temperature Variations in Air-Cooled Cold Storage Rooms. T. A. STEEVES AND W. H. COOK, Nat. Res. Council, Ottawa, Canada. Canad. Jour. Res., *23*, Sec. F, No. 4: 253. 1945.

Temperatures prevailing at different points in an experimental cold storage room with false floor were found to be subject to wider variations when boxes were present in the storage. By forcing the air to pass through the stack such variations were reduced to approximately the same values as those prevailing in an empty room. Dunnage spacing also reduced variations but no significant differences were found when spacings were varied from $\frac{5}{16}$ inches to 2 inches. O.R.I.

47. ABC's of Plant Refrigeration. LEON BUEHLER, JR., Creamery Package Mfg. Co., Chicago, Ill. 18th Ann. Rpt. N. Y. State Assoc. Milk Sanit., pp. 17–30. 1944.

The elementary physical facts that are basic to understand mechanical refrigeration systems are presented together with certain applications to dairy plants. A.C.D.

48. Truck Refrigeration. H. WILEY KROTZER, Engineer, Pioneer Ice Cream Div., The Borden Co., New York, N. Y. Refrig. Engin., *50*, 4: 324. Oct., 1945.

The writer presents a brief resume of refrigerated truck transportation development stating that pay loads have been doubled while the transportation costs, cost of chassis and body, and the number of vehicles required have been halved compared to the situation of 15 to 20 years ago. Out of his years of experience in building refrigerated truck bodies, Mr. Krotzer recommends the making of the following analysis before buying such vehicles: 1.) What product do you wish to transport? a. Is the product precooled. b. Allowable temperature limits. c. Time required for breakdown and repair factor. *d. Heat loss through vehicle walls. *e. Heat loss through door openings. *f. Total heat loss plus factor of safety. 2.) Amount of load and size of body. a. Construction. b. Insulation. c. Package shape and system of loading. 3.) Refrigeration analysis. a. Total Btu. required plus factor of safety. b. Portable or plant supply. c. Factor of safety in system chosen. d. Proposed time in years of operation. e. Investment and yearly depreciation both of refrigeration system and truck body. f. Care and upkeep. (Double your original estimates, since new installations get old faster than you realize.) g. Actual cost per day. (Be realistic about this—don't deadhead this operation just to get good costs.) h. Total costs per day, per year, or total cost per ton, on unit hauled. i. How does your driver like the vehicle and will he be better off in terms of salary or easier work by this system? 4.) If possible, consult an experienced transportation specialist and have him check your figures and make suggestions.

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* A more appropriate terminology would be heat gain when refrigeration is under consideration.

Emphasis is placed upon the role of body design to take care of factors of inherent moisture conditions, rusting of metals, rotting of wooden members, and nullification of insulation through moisture, rotting, fungus growth, settling, cracks, and frost deposits. L.M.D.

49. Vermin Control in Dairy Plants. PAUL W. REED, Captain SnC., before the Annual Meeting of the American Butter Inst., Chicago, Ill. Nov. 30, 1944. South. Dairy Prod. Jour., *37*, 3: 74, 80. March, 1945.

This paper is limited to the cockroach and the rodent. Both are the products of filth. Therefore, the best control is good housekeeping which denies the pest food and harborage.

The cockroach is nocturnal and hides during daylight hours near sinks, behind wall cabinets, under cracks in walls and floors and in dark food storage spaces. A permanent method of control is to seal these openings.

Sodium fluoride is the most effective poison against roaches. To be completely effective all possible hiding places must be sprayed or dusted weekly until all roaches disappear. Since sodium fluoride is a poison, it should be used intelligently. The Army uses the powder which has been colored green.

Powdered borax is less effective. A 1.5 per cent solution of boric acid in water distributed so as to be available for drinking is fairly satisfactory. Trapping is not successful as a rule. Baits containing phosphorus or arsenic may be used with success. Pyrethrum sprays are effective if they contact the roach.

Fumigation with hydrocyanic acid gas or methyl bromide is very effective against roaches and rodents, but the building must be taken out of use for 48 to 72 hours. Fumigation is much more expensive and should be done only by experienced personnel. DDT is no more effective than sodium fluoride for roaches.

Rats are very destructive and are also disease carriers. Control measures used against rodents are classified into two general classifications: the preventive procedures which include good housekeeping and rat proofing of buildings, and the destructive procedures which include baiting, trapping and fumigation.

Rat proofing is an engineering problem. The most successful poisons are barium carbonate and red squill which are used by mixing thoroughly one part poison with sixteen parts of hamburger, oatmeal, cheese, fresh grain or other bait. The baits are most attractive to the rat when about a teaspoonful is wrapped in paper. Prebating is considered desirable.

The campaign must be quick and decisive to avoid suspicion and refusal of baits by the rats. Baits should be removed the following morning and baiting repeated after a two-week rest period with different bait.

The snap trap is preferred in trapping. However, the cage trap may trap large numbers. The bait on the trap must be large, attractive and securely attached to the trigger. Fifty or a hundred traps all set along the normal runways of the rodents, and if the baits do not attract the rats, the traps should be camouflaged. F.W.B.

50. **Warning Agents in Odorless Refrigerants.** R. J. THOMPSON, Kinetic Chemicals, Inc., Wilmington, Del. Refrig. Engin., *50:* 4, 313. Oct., 1945.

Odorants and irritants each have undesirable qualities which render them unsatisfactory as warning agents for detecting odorless refrigerant leaks. The necessary requirements for a warning agent whether odorant or irritant are given as: (1) Should be distinctive, powerful, non-flammable, and non-toxic; (2) Must not be corrosive to copper, brass, tin, lead, zinc, cast iron, steel, aluminum, or any other metal under the conditions of machine operation; (3) Must be stable in the presence of the refrigerant mineral oil, and moisture at temperatures of operation of the machine; (4) Must be soluble in the liquid and vapor phases of refrigerant, in mineral oil, and in mixtures of refrigerant and oil; (5) It should have such physical properties—namely, boiling point, melting point, vapor pressure, and solubility—that its presence at any moment in all parts of the refrigerating system will be assured; (6) It should require a relatively small concentration in refrigerant; (7) It should be low-priced and easy to manufacture. Mr. Thompson, however, does not advise the use of odorants or irritants in connection with Freon-charged apparatus. The high molecular weights of the Freons, particularly Freon 11 and Freon 12, cause them to have much lower leak rates than other refrigerants. The use of the halide lamp is recommended for Freon leakage detection with the warning that the pump-up type, or one with a brass burner does not give sufficiently rapid color change of the flame. Leaks of as low as one ounce of liquid per month, 0.06 per cent Freon vapor in air, are readily detected by this method. L.M.D.

51. **Job Safety Training Manual.** KENNETH L. FAIST AND STANTON M. NEWKIRK, Nat'l Foremen's Inst., Inc., Deep River, Conn. 1945.

Complete details are given for instituting instruction in job safety of foremen in connection with the establishment of a safety program. Largely based upon the techniques of Job Instruction, Job Relations and Job Methods Training which have been developed during war period by the Training Within Industry (TWI) branch of the War Manpower Commission. The way to safety is based on four steps: (1) Analyze the Job; (2) Spot the Danger; (3) Act to Correct; (4) Check Back. These four steps of Job Safety Training should be used before giving a new man instruction, before giving an experienced man instruction, and when looking for danger spots to eliminate. Anyone can "spot the danger" AFTER the accident but if the educational aspect of the Job Safety program is carried out, accident incidents will be greatly reduced to the category of "unavoidable" accidents and man-hour work less minimized. L.M.D.

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CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

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Coulter, S. T.

Doan, F. J.

Dorsey, L. M.
Downs, P. A.

Ely, Fordyce
Erb, J. H.
Espe, D. L.

Frazier, W. C.
Fuller, S. A.

Glick, D. P.
Goss, E. F.

Henderson, J. L.
Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Lucas, P. S.

Marquardt, J. C.
Mueller, W. S.

Price, W. V.
Pyenson, Harry
Reece, Ralph P.
Roberts, Henry E.

Salisbury, G. W.

Thomsen, L. C.
Trimble, C. S.
Trout, G. M.

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Certified Milk
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Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
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Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
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Journal of Infectious Diseases
Journal of Milk Technology
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Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
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Prussian Dairy Research Institute, Kiel, Germany

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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

52. **Control of Dust Contamination in Agar Pour Plate Analysis of Milk.** G. C. GAMBRELL, Health Officer, Lexington, N. C., and MORRIS OSTROLENK, State Board of Health, Raleigh, N. C. Food Res. *10.* No. 4: 303. July–August, 1942.

A comparison of the degree of contamination of agar plates poured under unfavorable laboratory conditions with those poured under favorable conditions revealed a 17.2 per cent contamination in the first instance and a 3 per cent contamination in the second. F.J.D.

BUTTER

53 **Vitamin A in Butter.** Bureau of Dairy Indus., U.S.D.A. Mis. Pub. 571, July, 1945.

Twenty State Experiment Stations and the U. S. Bureau of Dairy Industry cooperated in this study to determine the vitamin A potency of butter produced and marketed in different seasons and different regions. The need for the study was indicated by the Food and Nutrition Board of the National Research Council. Some 3,500 samples of butter were analyzed from 14 states that produced 64 per cent of the butter in the United States.

"In all but one state there was a distinct difference between the vitamin A potency of butter produced under summer-feeding conditions. About 36 per cent of all the creamery butter is 'winter' butter, and it has an average vitamin A potency of approximately 11,200 International Units per pound. About 64 per cent of all creamery butter is 'summer' butter, and it has an average vitamin A potency of approximately 18,000 International Units per pound." The average for all creamery butter is about 15,000 I.U. per pound. When people eat the prewar per capita consumption of 18 pounds per year, butter furnishes 740 I.U. per capita per day or 14.8 per cent of daily requirements.

The effect of storage and handling on the vitamin A potency of butter was negligible, as reported by seven states that studied this point. In four states that studied butter on the retail markets, it was found that the vitamin A potency compared favorably with the vitamin A potency of the butter produced.

"Milk produced under winter-feed conditions has an average vitamin A potency of 1,140 International Units per quart (4 per cent fat basis) and that produced under average summer-feeding conditions has a potency of 1,800. The average potency of the total supply of milk which disappears annually for domestic consumption is 1,530 International Units per quart.

When the disappearance of milk for domestic consumption in all forms averages 1.052 quarts per capita per day, as it did in 1941, milk and milk products furnish the total population of this country about 1,600 International Units of vitamin A per capita per day, or nearly one-third of the recommended allowance."

The vitamin A potency of milk and butter is dependent upon the carotene content of the diet of the cows. A.C.D.

CHEESE

54 **A Grating Type Cheese.** C. D. DAHLE AND G. H. WATROUS, JR. Pa. State Col., Nat. Butter & Cheese Jour., *36,* No. 11: 44. Nov., 1945.

A new method of making a hard cheese for grating is described. The cheese develops a high, piquant flavor in approximately 10 months of curing. The characteristic flavor is caused by free fatty acids produced by the lipase of raw skim milk acting on the fat of homogenized, pasteurized, 20% cream. The cheese is made from a skim milk and cream mixture with 2% fat. The curd is made like stirred-curd cheddar but is cut finer, cooked to 115° F. and is only partially salted in the vat. It is pressed in Longhorn hoops and then is dry salted, oiled and rubbed with black pepper while curing at 60° F. and not over 80% relative humidity. The cheese contains about 27% fat, 27% moisture, 5 to 6% salt and has a pH of 5.2–5.7. W.V.P.

55 **The Trend Toward Bigger Plants.** ANONYMOUS. Natl. Butter & Cheese Jour., *36,* No. 9: 36. September, 1945.

Mr. Donald E. Hirsch in the article entitled "Farmers' Co-operatives and the Trend Toward Large Scale Dairy Plants," Misc. Rep. #80, Coop. Res. & Service Div., Farm Credit Administration, March, 1945, points out that large plants in general can utilize milk solids more efficiently than small plants. They achieve greater flexibility and have definite advantages in manufacturing, marketing and research not enjoyed by smaller organizations. Graphs are presented to illustrate the trends of increasing production per plant in the butter and chesse industries. W.V.P.

CHEMISTRY

56 **A Simple Fluorometric Method for Thiamin in Milk.** A. Z. HODSON, Res. Lab., Pet Milk Co., Greenville, Ill. Food Res. *10,* No. 4: 351. July-August, 1945.

A simplified fluorometric method for thiamin in milk based on the procedure of Hennessey and Cerecedo, but with the omission of the base-exchange step is presented. The method appears to be quick and reasonably accurate for milk and some other dairy products as judged by comparisons with two biological methods employing *Phycomyces blakesleeanus* and *Streptococcus salivarius* S20B.

Results with the *Phycomyces* method were high for evaporated milk which was interpreted as indicating that the heat destruction of thiamin in this product is a simple cleavage into the pyrimidine and thiazole components which are biologically active for the mold. F.J.D.

ICE CREAM

57. What the Ice Cream Industry is Doing About Frozen Foods.
Anonymous. Ice Cream Field, *46,* No. 4: 16. 1945.

This is a report of a recent survey, attempting to ascertain the place of frozen foods in the ice cream industry. Various views are expressed in answer to questions regarding (1) the use of dealer ice cream cabinets, (2) installing display cabinets to merchandise frozen foods and package ice cream, (3) using the same delivery trucks and storage rooms for ice cream and frozen foods, (4) maintaining separate sales personnel for ice cream and frozen foods, and (5) restricting the number of frozen foods items distributed. W.C.C.

58. Merchandising Frozen Cooked Foods.
Donald K. Tressler, Technical Consultant, Ice Cream Field, *46,* No. 4: 68. 1945.

Some frozen cooked food specialty items appeared on the market prior to the war. During the war, several others were added to this list. Difficulty in distributing such products has been due to limited frozen food outlets, but chain grocery stores, super-markets, and department stores, it is stated, are now looking for other frozen cooked foods. Over forty frozen cooked foods are listed as now on the market.

The author cautions against freezing any cooked food, and recommends preliminary freezing and storage tests at various temperatures before attempting to produce such items on a commercial scale. Once a satisfactory recipe has been found, it should not be changed, unless the new recipe has proved satisfactory under thorough testing. The importance of chemical, as well as bacteriological controls, is stressed. W.C.C.

MILK

59. Use of Plate Type Heat Exchanges in High-Temperature, Short-Time Pasteurization.
W. W. Cavanaugh, York Corp., York, Pa. Natl. Butter & Cheese Jour., *36,* No. 9: 38. September, 1945.

This is a detailed description of the design, operation, control and care of this type of pasteurizer and such auxiliaries as storage tanks, filters, clarifiers, homogenizers and separators. W.V.P.

60. Wartime Changes in Milk Distribution and in the Consumption of Milk, Cream, Butter and Oleomargarine in Vermont. THURSTON M. ADAMS. Vt. Agr. Expt. Sta. Bul. 527. 30 pages. Oct., 1945.

The bulletin is based on information obtained for the most part from personal interviews in June, 1944, with 1,150 housewives in Burlington, Vermont, and in two nearby villages. Information contained in 112 usable replies to questionnaires sent to all milk dealers in Vermont is also included.

If a cent a quart could be saved over three-fourths of the housewives having doorstep delivery of milk would be willing to have every-other-day retail milk delivery continued after the war. In June, 1944, store purchases of milk in Burlington amounted to 42 per cent. Low rental areas purchased more milk at stores than those living in high rental areas. Family milk supply purchased at stores tended to increase with alternate-day delivery. Nearly all the milk dealers in Burlington and Rutland (Vermont's two largest cities) favored every-other-day delivery. In the smaller communities, many were opposed. Of the 112 dealers reporting, 53 per cent favored continuation, 38 per cent were opposed and 9 per cent were undecided at the time they replied. A savings resulted from a reduced number of distributors in the market aside from every-other-day delivery. The number of dealers serving the Burlington area declined about one-half between 1942 and 1944. This decline in the number of milk distributors is but an acceleration of a long-time trend in Vermont and in many other markets. Mileage savings result when fewer delivery trucks serve an area.

While milk consumption in the homes of the areas studied was high and had experienced a war-time increase, it was below the level recommended for adequate nutrition. While nearly all the families studied had fresh milk, only about one out of every five families purchased cream. About one half pound per person of butter and margarine were used weekly in the area studied. About two-thirds as much oleomargarine as butter were purchased by consumers in Burlington and Essex Junction, while in the small rural community of Shelburne, they were buying nearly twice as much oleo as butter. Butter consumption declined and oleomargarine consumption increased more under war-time conditions, in the areas studied than in the country as a whole. In a large measure those families who had shifted to the use of margarine when butter was in short supply had returned to the use of butter when it again became available.

Only a small portion of the new war-time users of margaine expressed a conviction that they would use the product in the post-war period. Nearly one-third of the new war-time users of margarine not planning to use it after the war indicated that while they preferred butter, they had no particular dislike for margarine. H.P.

61. Certain Defects in Market Milk By-products. R. J. RAMSEY, Ramsey Laboratories, Cleveland, Ohio. The Dairy World, *24*, No. 2: 21. July, 1945.

The author discusses, in some detail and from the viewpoint of the plant operator, many troublesome problems such as: poor keeping quality, lipase action, cream plug, cream line and air incorporation in the production of bottled cream; factors influencing deterioration of frozen cream; sediment, flavor, soapiness and curd tension of homogenized milk; production of superior buttermilk and cottage cheese; homogenizing pressure, temperature of pasteurization, feathering, sediment and whipping qualities of homogenized cream; and sediment and sweetening problems of chocolate milk. F.J.D.

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CONTENTS

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Erb, J. H.
Espe, D. L.
Frazier, W. C.
Fuller, S. A.
Glick, D. P.
Goss, E. F.
Henderson, J. L.
Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Lucas, P. S.

Marquardt, J. C.
Mueller, W. S.

Price, W. V.
Pyenson, Harry
Reece, Ralph P.
Roberts, Henry E.

Salisbury, G. W.

Thomsen, L. C.
Trimble, C. S.
Trout, G. M.

Waugh, R. K.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.

Yale, M. W.

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Journal of Experimental Medicine
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Journal of Heredity
Journal of Infectious Diseases
Journal of Milk Technology
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Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

62. **A Swab-slant Test for Equipment Sanitation and Its Correlation with a Standard Method.** M. C. JAMIESON AND K. H. CHEN, Department of Animal Pathology and Bacteriology, The University of Manitoba, Winnipeg, Canada. Jour. Milk Technol., *8*, No. 3: 134–139. May–June, 1945.

The authors prepared wooden applicators with 0.05 g. of good absorbent cotton and packaged and sterilized in glassine bags.

Sterile water was prepared in screw-scapped vials of 40 ml. capacity for moistening the swabs. The material tested was swabbed and streaked on suitable culture medium at the time the swab was taken.

The results obtained by the investigators lead to their conclusion that the swab-slant test correlated very closely with standard procedure as outlined in Standard Methods, except that the slants and plates were incubated at 25° C. for 48 hours. Colonies were counted with the aid of a Quebec Colony Counter.

The "swab-slant test" offers possibilities as a simple and practical test for checking the sanitization of dairy food handling and beverage dispensing equipment. H.H.W.

BUTTER

63. **Production of Butter from Neutralized Sour Cream.** E. W. BIRD, Professor of Dairy Industries, Iowa State College, Ames, Iowa. Milk Plant Monthly, *34*, No. 11: 39. 1945.

Proper reduction of cream acidity and production of uniform quality butter from processed cream is dependent upon paying attention to certain details and principles such as (1) correct sampling of the cream; (2) correct manipulation of the acidity test; (3) exact knowledge of the neutralizing factor of the material employed; (4) knowing the exact weight of the cream in the vat; (5) reduction of the cream acidity to a pH between 6.6 and 7.2; (6) use of neutralizing charts; (7) accurate weighing of neutralizers to one-tenth of a pound. G.M.T.

64. **Why Color of Butter is Standardized.** FRED H. ABBOT, University of California, Davis. Natl. Butter and Cheese Jour. *36*, No. 10: 56. Oct., 1945.

The shade of butter color depends on the cow, her breed, feed, and period of lactation. Green feeds, cows of the Channel Island breeds, and milk produced in early stages of lactation, all produce more highly colored butterfat. Natural color ranges from deep yellow to canary. This color must be standardized to the high level for uniformity in packages. W.V.P.

65. Changes in Creamery Costs During the War. E. Fred Koller, University of Minnesota, St. Paul. Natl. Butter and Cheese Jour., *36*, No. 10: 37. Oct., 1945.

Costs of operating seventy-five identical Minnesota co-operative creameries in 1940 and 1944 are summarized.

Expense	Cents per pound of butter made*		Increase or decrease, per cent
	1940	1944	
Labor and management	0.808	1.193	+ 47.6
Manufacturing	1.288	1.511	+ 17.3
General and administrative	0.312	0.414	+ 32.7
Interest on loans	0.018	0.015	− 16.7
Total	2.426	3.133	+ 29.1

* Pounds of butter made include the butter equivalent of fat sold in fluid cream.

W.V.P.

CHEESE

66. The Retention of Nutrients in Cheese Making. O. R. Irvine, L. R. Bryant and W. H. Sproule, Ont. Agr. Coll., Guelph, Ontario, and S. H. Jackson, A. Crook, and W. M. Johnstone, Hosp. for Sick Children, Toronto, Ontario. Sci. Agr., *25*, No. 12: 817; 833; 845. Aug., 1945.

I. The Retention of Calcium, Phosphorus, and Riboflavin in Cheddar Cheese Made from Raw Milk.

The efficiency of the cheddar cheese-making process with regard to the retention of these nutrients was studied in 12 trials. The effect of ripening the cheese at 40° and 58° F. upon the stability of riboflavin during 12 months was also studied.

About 61 per cent of the calcium and 53 per cent of the phosphorus originally present in the milk were retained in the cheese. These proportions were subject to small variations but did not appear to be related to minor variations in the method of cheese making nor to seasonal changes.

About 23 per cent of the riboflavin originally present in the milk was retained in the cheese although in this case the results were somewhat less consistent. Riboflavin values appeared to decline markedly during the first two months of ripening but later rose to the values found in the fresh cheese.

Mineral losses were almost entirely accounted for in the whey but there was some loss of riboflavin which could not be accounted for and which may have resulted from photochemical destruction during manufacture.

Samples of milk, whey and cheese secured from four factories representative of four soil types gave results similar to those in the controlled experiment. This was also true of cheese displaying defective flavor qualities.

II. The Effect of Pasteurization of the Milk upon the Retention of Calcium, Phosphorus and Riboflavin in Cheddar Cheese.

In each of six experiments approximately 1800 lb. of milk was divided into three lots, one of which was made into raw milk cheese while the second and third were made into cheese after the milk was pasteurized by the holder and high-short methods respectively. The efficiency of calcium retention was not affected by pasteurization, the average values being 61 to 63 per cent. Pasteurization of the milk resulted in a slightly improved retention of phosphorus, the three mean values being 53.1, 57.0 and 55.5 per cent, respectively. Neither the retention efficiency nor the stability of riboflavin during ripening was affected by pasteurizing the milk.

III. The Calcium, Phosphorus and Riboflavin Contents of Cream, Cottage, Brick and Blue Cheese.

Four lots of each of these varieties were manufactured under controlled conditions. Cream cheese of about 50 per cent total solids content contained 84.4 mg.% Ca, 86 mg.% P and 280 μg. riboflavin per 100 g. of cheese. Corresponding values of cottage cheese of about 20 per cent total solids content and 2.6 per cent fat were: 85 mg.% Ca, 146 mg.% P. and 288 μg. riboflavin.

Since whole milk was used in the manufacture of the brick and blue cheese, retention values were calculated on the basis of the original milk. In brick cheese 57.7 per cent of the calcium, 58.7 per cent of the phosphorus and 27.4 per cent of the riboflavin were retained. The corresponding values for blue cheese were 46.2, 43.3 and 30.1, respectively. Riboflavin was found to be stable in cream and cottage cheese when samples were stored at 40° F. for 7 and 14 days respectively. It also appeared to be quite stable during the ripening of brick and blue cheese. O.R.I.

CHEMISTRY

67. The Purification and Crystallization of Rennin. N. J. Berridge. Natl. Inst. Res. in Dairying, Univ. of Reading (Eng.). Biochem. Jour., *39*, No. 2: 179–186. 1945.

The author summarizes the experience gained in preliminary purification experiments and describes the preparation as well as some properties of the rennin crystals which were finally obtained.

"Rennin crystals are of very low solubility in water and can be dried without decomposition. Rennin is probably a protease, acting optimally on haemoglobin at pH 3.7." A.O.C.

68. **Use of Enzyme in Riboflavin Determination. Free and Combined Riboflavin.** L. Rosner, E. Lerner and H. J. Cannon, Lab. of Vitamin Technology, Chicago, Ill. Jour. Indus. Engin. Chem., Analyt. Ed., *17,* No. 5: 778–779. Dec., 1945.

In the fluorometric determination of riboflavin on many natural products by a method involving adsorption on Floristil without preliminary enzyme digestion, low results were obtained due to nonadsorption of a fraction of the riboflavin on Floristil. Evidence is presented that nonadsorption of part of the riboflavin is not due to physical factors alone but also to a difference in character between adsorbed and nonadsorbed riboflavin. The nonadsorbable or "combined" riboflavin is converted to the adsorbable form by enzyme. Results are given for determinations on various foods including dried skim milk and whey. B.H.W.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

69. **Condensed Milk By New Method.** John C. Burtner, Ice Cream Field, *26,* No. 4: 26. Nov., 1945.

A brief review is given of a method of making condensed milk as developed by Dr. G. H. Wilster, professor of dairy manufacture at Oregon State College. In short, the method consists of heating milk continuously in a preheater, then circulating it through a Vacreator ("vacuum pasteurizer") until the desired concentration is reached. When the Vacreator is used for condensing, a relatively high preheating temperature is employed, from 200° to 205° F., in addition to the elimination of steam injection in the equipment when it is used as a condenser.

This equipment has successfully been used in pasteurizing cream for butter manufacture and according to the report, Wilster was the first to use it successfully to pasteurize ice cream mixes. He has also extended its use to the condensing of milk. Details of this research, it is stated, are to be published in a bulletin entitled "The dual use of the Vacreator for condensing milk and pasteurizing ice cream mix." W.C.C.

70. **Ethyl Gallate and Abscorbic Acid as Antioxidants in Dried Whole Milk.** J. D. Findlay and J. C. D. White. Biochem. Jour., *39,* No. 2: XXXIV. 1945.

"Milk powder was made from one batch of full-cream milk with and without the addition of very small amounts of ethyl gallate and ascorbic acid. Both these compounds lengthened the storage life of the powder very materially, ethyl gallate causing a greater extension of storage life than

ascorbic acid. During storage the concentration of ethyl gallate in the powder remained unchanged, but the ascorbic acid gradually decreased."
A.O.C.

71. **Dried Milks—.** GEORGE E. HOLM, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Dept. of Agriculture, Washington, D. C. Milk Plant Monthly, *31*, No. 8: 23, 52, 54, 65–67, 69. 1945.

Other than malted milk, the manufacture of dried milks is a relatively recent development in the dairy industry. Production of dried milk has increased markedly since 1934. Three principal methods are used in drying milk, namely, spray, atmospheric drum and vacuum drum processes. Dried milks made by the spray process are finely divided, very soluble and hygroscopic. The atmospheric drum process is used mainly in the drying of skim milk and buttermilk. Dried milks made by this method are practically nonhygroscopic. The vacuum drum process is essentially a roller process enclosed under partial vacuum during the drying operation. The products produced by this process resemble those made by the spray process in solubility, color, flavor and hygroscopicity. Inasmuch as the spray- and vacuum-dried milks have a great avidity for moisture, they must be packaged against absorption of moisture from the atmosphere. Dried whole milk deteriorates relatively rapidly when packaged in contact with air. In order to prevent spoilage through oxidation of the fat, this product is packaged in an atmosphere of inert gas, usually nitrogen. The principal types of off-flavors found in dried milks are cooked, which develops during manufacture; staleness, rancidity and tallowiness which develops during storage. Defects of discoloration, caking or fishiness may develop if the moisture content of the product exceeds 2 to 3%. Dried milk gas-packaged and stored at 75 degrees may be expected to remain in good condition for at least a year. Costs of drying milks by different methods and processes are not available, but estimates place the cost of drying skim milk at from 2 to 3 cents per pound of dried product. Data are presented showing the nutritive value of dried milk, as well as uses, definitions and standards. The author has included sources of information as well as listing some manufacturers of dried milks and milk driers. G.M.T.

DISEASE

72. **Comment on How to Handle the Mastitis Problem.** C. S. BRYAN, School of Veterinary Medicine, Michigan State College, East Lansing, Mich. Jour. Milk Technol., *8*, No. 3: 157–161. May–June. 1945.

The author classifies mastitis into acute, marked by swelling, pain and the production of abnormal milk, while acute systemic type is associated with a sick cow and frequently is fatal. Again udder troubles may be divided into noninfectious, due to chilling, bruising and injury to the udder during the milking operation; infectious mastitis is usually associated with Streptococci, Staphylococci and *Escherichia coli.*

An attempt to determine the cause of mastitis should preclude any method of treatment. The following steps are summarized briefly:

1. The use of any test that will reveal the abnormal condition of the udder.
2. Prompt treatment before extensive changes take place in the udder.
3. Precautions should be used in treating cows with acute mastitis.
4. Better results are obtained in treating cows at the end of lactation or during the dry period.
5. Abnormal milk should never be used for human consumption.
6. Immunity is not conferred upon any cow as the result of treatment so that the animal becomes free from infectious agents. On the other hand, any program based on good practical sanitary practices to prevent re-exposure of the recovered case is desirable. H.H.W.

73. **Can a Differential Germ Test with a Leucocyte Count Be Used in the Control of Mastitis?** N. O. Gunderson, Commissioner of Health, and C. W. Anderson, Director of Laboratories, Rockford, Ill. Jour. Milk Technol., *8*, No. 3: 147–152. May–June, 1945.

The authors suggest the following procedure in the control of mastitis: 1. Determine the type of organisms present in the milk; 2. Classification of milk into 4 classes based on the presence of streptococci and leucocytes by microscopic examination. (a) Leucocyte count 500,000 per ml.; (b) 500,000 to 1,000,000; (c) 1,000,000 to 7,500,000; and (d) over 7,500,000 per ml. Additional information may be useful in differentiating saprophytic streptococci from *Streptococcus agalactiae* by the inability of the former to produce ammonia in arginine broth. Polyvalent and type-specific antisera for identifying bovine streptococci may be useful. The following conclusions were made: 1. Leucocytes are directly related to stage of lactation, disease and age of the cow; 2. The "differential germ test" may be useful in the control of infectious mastitis; and 3. Good herd management and sanitary practices must be carried on concurrently under the laboratory examinations.

H.H.W.

74. **The Effect of Penicillin on Staphylococci and Streptococci Commonly Associated with Bovine Mastitis.** E. G. Foley, S. W. Lee and G. A. Epstein, Wallace Laboratories, Inc., New Brunswick, N. J. Jour. Milk Technol., *8*, No. 3: 129–133. May–June, 1945.

Streptococcus agalactiae (Lancefield's group B), *Strep. dysagalactiae, Strep. zooepidemicus* (Lancefield's group C) and *Staphylococcus ameus* constitute the main group of microorganisms associated with bovine mastitis.

The authors concluded that penicillin shows considerable promise in the treatment of mastitis caused by groups B and C streptococci and staphylococci.

Approximately 100–200 Florey Units of penicillin per mg. and liquor filtrates of *Penicillium notatum* containing 25 to 80 Florey Units per ml. were diluted and used against the test organisms grown in defatted pasteurized milk.

Their susceptibility was compared by seeding 0.1 ml. of 1–10 dilution of 18-hour cultures in 5 ml. amounts of brain heart broth containing different dilutions of penicillin and then plated on proteose tryptone agar. The presence or absence of growth was noted after 23 hours incubation at 37° C.

Streptococcus agalactiae, Strep. dysagalactiae, Strep. zooepidemicus and *Staphylococcus ameus* were highly susceptible to small amounts (1–2 Florey Units) of penicillin. H.W.W.

FOOD VALUE OF DAIRY PRODUCTS

75. Changes on Storage in the Biological Value of the Proteins of Dried Skim Milk. K. M. Henry and S. K. Kon. Biochem. Jour., *39*, No. 2: XXVI. 1945.

While a certain amount of information is available on changes in the biological value of the proteins of various food products brought about by heat treatment, little is known about the effects of storage; nothing, as far as we are aware, regarding dried milk.

Since 1941 we have examined on several occasions by the method of Mitchell on groups of 12 rats the biological value of the proteins (nitrogen) of a sample of spray-dried skim milk manufactured in 1939, and since stored at room temperature. The results are given below.

Test No.	*Length of storage (months)*	*Biological value*	*True digestibility*
1	19	88.5	89.8
2	40	81.3	87.6
3	43	80.3	96.9
4	49	75.0	88.1
5	54	71.1	88.1

Normally, dried skim milk would not be stored for such prolonged periods but it is generally accepted as a product of great stability and our observations may have a practical bearing. They are in agreement with similar findings regarding the effects of storage on the proteins of maize and of soya bean. It should be noted that the true digestibility of the proteins of the milk remained unimpaired. Further work on the nature of the changes is in progress. A.O.C.

ICE CREAM

76. Lecithin in Chocolate Ice Cream. B. I. Masurovsky, Research Editor, Ice Cream Trade Jour., *41,* 10: 48. Oct., 1945.

Lecithin from soybeans is a well-known ingredient used in candy as a protective colloid to stabilize and insure uniformity in the finished product. Its use in ice cream has been limited to that found in egg yolk, buttermilk powder and cream, and that used in chocolate coatings for ice cream bars. Commercial lecithin is a material extract of soybeans, prepared in concentrated form and added to an oil in such quantities as to facilitate handling and application. Recently quick emulsifying lecithin has been developed which is miscible in water and is easy to handle in the preparation of an ice cream mix. About 0.15 per cent is the maximum amount used in ice cream. It is added to the mix by mixing with sugar and adding to the mix at 100° F. before pasteurization. The amount of lecithin for ices and sherbets is about 0.2 per cent.

When lecithin is used in combination with a stabilizer in a chocolate ice cream mix, it is advisable to reduce the amount of stabilizer about 0.1 per cent.

The lecithin when used in a chocolate mix acts as an emulsifying agent for the cocoa fat. It also helps to dissolve the cocoa particles by acting as a wetting agent. Chocolate ice cream containing 0.1 per cent lecithin and 0.4 per cent gelatin was found to have a smoother texture and smaller air cells in the foam from the melted ice cream than that which contained 0.5 per cent gelatin and no lecithin. W.H.M.

77. 1940 vs. 1945 Costs. Ice Cream Trade Jour., *41,* 11: 38. Nov., 1945.

Joseph Morrissey of the DeCoursey Creamery Co., Kansas City, Kansas, had made a study of the ice cream ingredient costs in his area in 1940 and 1945. The comparisons show that there has been an increase of 8 to 278 per cent in the cost of the various ingredients. Butter fat and other milk products have more than doubled in price. Other costs such as labor and taxes have also increased during the five-year period. W.H.M.

78. Ice Cream at the Fountain of Tomorrow. Ice Cream Trade Jour., *41,* 11: 30. Nov., 1945.

In order to meet the increased demand for ice cream equipment, manufacturers are designing larger soda fountains with improved facilities for handling larger volumes of ice cream. New features will include improved counter designs, better balanced and more comfortable stools, better leg room, greater accessibility of the various departments and improved appearance. Trained personnel will be needed to operate soda fountains. It is estimated that from 100,000 to 500,000 new people will be needed to fill up the ranks now understaffed and to take care of expected increases in business. The Ice Cream Merchandising Institute is now working on a project which will help train fountain employees. W.H.M.

79. **The Influence of Soda Fountains on Ice Cream Sales.** Ice Cream Trade Jour., *41,* 11: 28. Nov., 1945.

The Ice Cream Trade Journal has tabulated the gallons of ice cream manufactured in each state in 1940 and the number of soda fountain installations and found that there is a very close relationship between the two. Of the ten leading states in ice cream production, nine of them were leaders in the number of soda fountains in operation. W.H.M.

80. **How the Food Chains Now Sell Ice Cream.** Ice Cream Trade Jour., *41,* 10: 54. Oct., 1945.

Seventeen per cent of the food chain stores which replied to the Ice Cream Trade Journal's survey indicated that they were now selling ice cream. At present the price ranges from 12 to 25 cents per pint and the volume per store ranges from 2,500 to 14,600 gallons per year. W.H.M.

81. **Forty Per Cent of the Food Chains Plan to Sell Ice Cream.** VINCENT M. RABUFFO, Editor. Ice Cream Trade Jour., *41,* 10: 50. Oct., 1945.

Replies to questionnaires sent to the leading food chain stores in 20 states by the Ice Cream Trade Journal indicate that 40 per cent of the reporting chains plan to sell ice cream; 17 per cent reported that they were already selling ice cream and 43 per cent professed no interest in the sale of ice cream.

A markup ranging from 10 to 30 per cent above the cost price was mentioned, with a majority of the chains, 67 per cent, indicating that they planned a markup of 20 to 25 per cent. This proposed markup is considerably below that used by present-day dealers, whose markups frequently range as high as 60 to 75 per cent.

Most chains favored the buying of ice cream from established manufacturers, rather than making their own. Fifty-eight per cent plan to use their own ice cream cabinets.

At present the pint- and quart-sized packages are most popular; however, other sizes including single gallons, or gallons broken into 8 pints or 4 quart units have been suggested.

Four of the leading food chain stores operate more than eleven thousand stores; should these stores and the thousands of others sell ice cream, they would provide an outlet for a large volume of ice cream. W.H.M.

MILK

82. A Time and Equipment Comparison Between the Methylene-blue and Direct Microscopic Tests.

K. M. MASON, Division of Sanitation, Department of Health, Peoria, Ill. Jour. Milk Technol., *8:* No. 3, 145–146. May–June, 1945.

Milk sanitarians and dairy technologists are interested in methods that are applicable to their particular situation. Obviously, any method that will determine quickly and accurately the quality of the milk from a bacteriological standpoint is desirable. The cost of additional equipment, extra hours of labor and clerical services should in the opinion of the authors, determine the method to be used.

A comparison of the methylene-blue reductase test and direct microscopic method were made on 100 samples of milk serving a population area of 150,000. The additional cost of $85.00 for direct microscopic examination was estimated while the laboratory time increased from 4.5 to 10 hours and clerical services from 1 to 3 hours per 100 samples of milk examined.

H.H.W.

83. The Babcock Test on Homogenized Milk.

L. M. LAMPERT AND J. H. BRANDON, Dairy Science Laboratory, Department of Agriculture, Sacramento, Calif. Jour. Milk Technol., *8:* No. 3, 140–144. May–June, 1945.

The comparison of the fat content of milk before and after homogenization at different pressures was made by using several modifications of the fat test. The regular Babcock test gave accurate results on homogenized milk as compared to untreated milk. Formaldehyde added to milk as a preservative gave a lower Babcock test than the unpreserved sample. Mercuric chloride enhanced a fat separation or ''oiling of'' which affects the fat test. The Pensylvania or Minnesota test are not satisfactory for testing homogenized milk.

H.H.W.

84. 14 Steps to Good Milk.

H. C. JACKSON AND EVERT WALLENFELDT, Dairy Department, University of Wisconsin, Madison, Wisconsin. Milk Plant Monthly, *34,* No. 8: 25, 30, 32, 34, 38, 62, 68, 70. 1945.

The authors describe and illustrate the fourteen steps which they deem necessary to the production of high quality milk. These steps are: (1) healthy cows; (2) careful feeding; (3) clean barns; (4) insect control; (5) well-made utensils; (6) utensils bacteria-free; (7) clean cows; (8) good milking methods; (9) clean milking machines; (10) clean utensils; (11) proper straining; (12) good milk house; (13) proper cooling; (14) enclosed truck.

G.M.T.

85. The Acid Milks: Their Potential Hazard. F. L. Wilson and F. W. Tanner, Department of Bacteriology, University of Illinois, Urbana, Ill. Jour. Milk Technol., *8:* No. 3, 127–128. May–June, 1945.

The milk sanitarians for many years have considered fermented milks safe from the public health standpoint. However, the U. S. Public Health Service has assembled sufficient evidence during the past few years in regard to disease outbreaks which have been traced to the consumption of fermented milks.

The authors made an extensive review of the literature and found many contradictory reports. A marked difference in the growth of acid-forming organisms and pathogens in milk acidified by the addition of acid as compared to milk fermented by normal acid-producing organisms is noted.

These investigators also studied the survival of the following test organisms: *Eberthella typhosa, Salmonella paratyphi, Sal. schottmulleri, Shigella dysenteriae* and *Shigella paradysenteriae,* (Flexner), when grown in sterile skim milk, acidified with lactic acid-producing bacteria and pathogens.

The following conclusions were drawn:

(1) The amount of acid varies in sterile skimmed milk produced by lactic acid-producing bacteria.

(2) The test organisms were destroyed more easily in acidified milk than in milk allowed to undergo natural fermentation.

(3) Larger numbers of pathogenic organisms survived in milk when the acidity increased gradually; perhaps the presence of acid-tolerant strains were present.

(4) The acidity of naturally fermented milks is not sufficient to destroy pathogenic organisms. Pathogens survived in milk with an acidity of 0.95 to 1.15 per cent (pH 3.9 to 4.2), an acidity of 0.50 to 0.86 per cent (pH 4.3 to 5.0) the organisms survived from two to nine weeks. H.H.W.

86. Butterfat Differentials. Stewart Johnson, Extension Economist and A. J. Mann, Extension Dairyman, University of Connecticut, Storrs, Connecticut. Milk Plant Monthly, *34,* No. 1: 30, 81–86. 1945.

Butterfat differentials paid to producers and butterfat differentials paid by dealers are described fully. Three primary objectives are to be considered in determining the producer butterfat differential; namely, (1) to provide for an automatic adjustment of rates so that the profitableness of low test and high test milk of cows will remain reasonably constant; (2) to arrive at such a rate which will equitably pay for milk according to what it is worth; (3) to establish a rate which will encourage production of milk having a desired butterfat test for the market. A survey of butterfat

differentials in several markets show that there is a variation in the butterfat differential, but exceeding in most markets four cents a pound, which rate applies in Connecticut. The rate should vary with the difference in the net returns obtainable from the milk of different tests and should be the basic principle to follow in determining butterfat differentials on class 2 milk. The authors believe that a varying rate of the producers differential on Class 1 milk, as the price level fluctuates is preferable to a fixed rate. Certain advantages are given for the fluctuating rate of butterfat differential.
G.M.T.

87. **Factors to Consider Before Adopting High-Temperature Short-Time Pasteurization.** C. F. WEINREICH, Director of Research, Cherry-Burrell Corporation, at the Milk Pasteurization Conference, Michigan State College, May 22 and 23, 1945. Milk Plant Monthly, *34,* No. 11: 25–28, 38. 1945.

The factors to be considered before adopting short-time high-temperature pasteurization center around the following five questions: (1) What is the trend in pasteurization? (2) Will the raw milk supply lend itself to high-temperature pasteurization? (3) How large an operation should be had before considering high-temperature pasteurization? (4) How shall the size of the unit be determined? (5) How do special operations such as separating, homogenizing and clarifying fit into this system of pasteurization. Data and illustrations are presented showing in considerable detail the various items which must be considered before adopting high-temperature short-time pasteurization. The example of a planned operating schedule is presented. In general 10,000 to 12,000 pounds of milk is the minimum amount usually handled economically by high-temperature short-time pasteurization. The slogan of "get it big enough" which seems to fit well with other types of equipment does not fit in with short-time operation because a plate unit can be expanded at any time by merely adding more plates. Special operations such as clarification and homogenization fit in readily with short-time pasteurization. G.M.T.

88. **Studies of Wartime Economies in Milk Delivery.** LELAND SPENCER, Professor of Marketing, Department of Agricultural Economics, New York State College of Agriculture, Ithaca, New York. Milk Plant Monthly, *34,* No. 11: 34, 46–47, 54–57, 84. 1945.

The prices paid for milk by consumers during the war increased much less than did the returns to producers. Despite increased price of milk to producers, an increase in wage rates of persons employed in milk processing and distribution, the spread between the price paid by the consumer and the amount received by the producer was reduced more than two cents a quart.

Two unusual developments made this possible: (1) payment of substantial subsidies by the federal government and (2) a remarkable lowering of milk distribution costs through economies brought about by certain restrictions of milk delivery service. The principle change in practice which made possible a saving of the cost of retail milk was the adoption of every-other-day service to retail consumers, which was put into effect by government order, primarily to conserve tires, gas and trucks during the war. Increasing the size of the route loads materially affected the delievery cost per quart of milk also. With the lifting of wartime restrictions on milk effective November 1, 1945, some questions receiving consideration by consumers, producers and distributors, and route men are as follows: (1) How many consumers prefer pre-war type of milk delivery service? (2) If wartime economies were abandoned, how much would the cost of delivery of milk to consumers be increased? (3) Would the resumption of pre-war delievery methods make the route man's job easier or more difficult, and how would it affect his earnings?(4) If the pre-war system is reinstated, how many more route jobs would be available? (5) Which system will yield the greater returns to producers? (6) If some of the economy practices are retained, what practical steps might be taken to insure their continuance? Indications are that if the pre-war type of delivery service is to be reinstated, that delivery of milk to retail consumers would be increased by 1.3 cents per quart and would reduce the earnings of route men about $3.00 a week. It would call for the operation of about ⅓ more retail routes. The attitude of the consumer will likely determine largely whether wartime practices will be retained or not. The retention of wartime milk delivery is of interest to the producer because it may have a significant effect on the volume of fluid sales and because of its effect on prices. The author believes that if more consumers on the retail milk routes have a strong preference for daily delivery and other services on the prewar pattern and are willing to pay the extra cost, more milk will be sold by reinstating that type of service. G.M.T.

MISCELLANEOUS

89. Tomorrow's Packages—an analysis. E. G. PENN, Riegel Paper Corp. Food Freezing, *I*, No. 1. Nov., 1945.

The first of a series of articles which will cover all of the materials that have been used and can now be used to do a protective job. When normal competition is once again fully under way in the food field, frozen foods will have to meet the "shelf life" of other processed foods. They must be able to stand up under months of storage without dehydration or deterioration. In evaluting a good frozen food package the protective qualities should be evaluated at 75 per cent, as against 25 per cent for sales appeal.

Sales-and-eye-appeal in packaging should be obtained through the advanced techniques of printing, design, display and advertising. The following requirements are becoming recognized as the ideal requisites of a protective material for frozen foods: 1. Moisture—vaporproofness. 2. Air-proofness and heat transfer. 3. Wet-strength. 4. Flexibility at low temperatures. 5. Closure strength. 6. Ease of filling and operational adaptability.

Glassine is presented as a packaging material possessing these attributes when it is manufactured in wet-strength quality obtained by treating the hydrated fibers with certain types of synthetic resins and applying a moisture-vapor proof lacquer or resin coating.

The author mentions that developmental work is underway to bring out a carton consisting of wet-strength glassine or greaseproof paper resin-laminated to carton-board stock, thus doing away with an outer wrap and an inner-liner or bag. Such a carton with foolproof locking ends would obviously effect considerable economy on the packaging line as well as meet the overall protective needs for frozen foods. L.M.D.

90. The Warehousing of Frozen Foods. PAUL B. CHRISTENSEN, Vice-Pres. and Chief Engineer, Merchants Refrigerating Co., N. Y. Food Freezing, *I*, No. 1. Nov., 1945.

The influence of the fast-developing frozen foods industry upon the cold storage warehousing industry has been to reverse the cooler and freezer space from 60 per cent and 40 per cent respectively to 60 per cent freezer and 40 per cent cooler space with prospect for even greater demand for freezer storage facilities. The refrigerated warehouse performs two very important and necessary functions in the flow of food commodities from the producer to the consumer. First, it absorbs production during harvest period and then gradually disperses it. Second, it serves as a consolidation and distribution point for these commodities. It is pointed out that even temperature at zero degree F. level is desired rather than fluctuating temperatures at below zero levels. The refrigerated warehouse can do its part by seeing to it that proper storage practices are followed, such as piling to permit air circulation, use of dunnage, and keeping packages from contact with outer walls. The ultimate goal is high quality of the food when it is placed on the table, as well as when it was packed. L.M.D.

91. Food Freezing, Vol. I, No. 1, Nov., 1945. Subscription rates: $4.00 per year; Canada, $4.50; Foreign, $5.00.

A new magazine makes its initial bow in the frozen foods industry. The publisher and editorial director is John T. Ogden. Food Freezing is dedicated to the task of promoting coordination of all of the frozen foods industry's specialized branches—processors, locker plant operators, distributors, warehouse men and transportation agencies—into one unified team, each member of which will be fully acquainted with his own problem and the other fellows' problems. This calls for data, news, ideas. Everything that makes the industry tick must be probed, analyzed, disseminated. The editor emphasizes the fact that quality cannot be frozen into foods—it must be there in the first place—a fact that new-comers either in the processing or distributing fields of the industry must realize to the fullest extent. Quality control fundamentals becomes a matter of education for a very large proportion of the industry. L.M.D.

92. **Principles of Industrial Process Control.** Donald P. Eckman, Engineering Department, The Brown Instrument Company. A division of Minneapolis-Honeywell Regulator Company. John Wiley & Sons, Inc., N. Y. 1945.

The author has brought together the many loose ends in the literature of automatic control in an attempt to provide a textbook for the student in chemical, metallurgical, mechanical or electrical engineering and as a reference for the industrial user of automatic control equipment. He had succeeded admirably in presenting in a logical manner the important laws of operation of industrial automatic control systems and their practical background of theory. The complete control system is divided into four elements: The measuring means, the controller mechanism, the final control element, and the process. It is emphasized that advancing technology in the industrial field is coming to depend more and more upon precision in processing, introducing standards which would have been thought of as impossible a few years ago. The elimination of manual control and the accompanying human element in processing has made for lessened expense, greater output, and a lowering of the inherent losses. Subjects treated are, The Art and Science of Control; Measuring Means; Modes of Automatic Control; Final Control Elements; Process Characteristics; Theory of Automatic Control; Quality of Automatic Control; Application Control Engineering; Automatic Control Systems; Maintenance of Exact Control. The text is illustrated with 178 figures key numbered by chapter number and sequence in the chapter. Many of the illustration figures are diagrams and the others are in the form of graphs. Numerous equations are keyed to the graphs by corresponding key numbers. The first chapter is preceded by a page of symbols, which are commonly used in the equations. A glossary of items peculiar to automatic control that is of great assistance in understanding the text follows the last chapter. Numerous references are included for each chapter. L.M.D.

93. **Transportation of Frozen Foods.** A. E. Huff, Manager, Transportation, Warehousing, Birdseye-Snider, Inc. Food Freezing, *I*, No. 1. Nov., 1945.

A review of developments in transportation of frozen foods since 1929 and some recommendations for the future. The early distribution of frozen food was largely local and offered no great problem except suitable truck body insulation. In later years with greatly increased business and multiplicity of products the long haul has entered the picture, and as yet is not very satisfactorily functioning in the case of frozen food protection. Cross continental movement by truck meets obstacles of state motor truck law restrictions which holds down development along that line. Rail transportation has been slow to utilize special refrigerator cars provided with super insulation and refrigeration to hold car temperature at zero degree temperature. Water-ice plus salt used to greatest extent, along with some solid carbon dioxide and mechanical refrigeration applications. Greater need for building refrigerator cars for the frozen goods industry. Two newcomers in this field are the airlines and the steamship companies. L.M.D.

94. **Insulation and Its Uses. Part II.** P. Edwin Thomas, Sales Mechanical Engineer, Washington, D. C. Refrig. Engin., *50:* 5. Nov., 1945.

The author explains a few fundamentals of physics—conduction, convection and radiation—concerned with heat transmission. He emphasizes the fact that a good insulator for cold-storage application must possess a low conductivity factor, and for all practical purposes surface effects (convection and radiation) may be disregarded when estimating heat transfer where such insulators are concerned. A possible 10% error of underestimation may be introduced under such circumstances. The efficacy of metal insulating materials for cold-storage insulation is questioned. The author's contention being that environmental air temperature is established largely because of radiation and convection thereby setting up the temperature gradient against which efficient insulation (low conductivity factor) must be built up to offset the conduction heat flow.

The greatest problem in the low-temperature insulation field is the one great one of "water." Nature is contantly at work to establish moisture equilibrium employing the agencies of water, moisture, and water-vapor. Three powerful means are in use for the above purpose, (1) wind or air-current pressures; (2) air-pressure differential; (3) vapor-pressure differential. These more or less combine to drive moisture into insulation and to overcome the destructive result from deposition of moisture in insulation material. The warm-side vapor barrier is the modern safeguard. A plea is presented for the manufacturers and purveyors of the bulk of reliable cold-storage insulating materials to develop and launch a program looking to the discovery and dissemination of such additional information to users and potential users of insulating materials as will safeguard the buyer's interest and thus advance the interests of the insulating manufacturers themselves. L.M.D.

95. Technical Tips on Fish Freezing in the Locker Plant. J. M. LEMON, Chief of Technical Research, Fish and Wildlife Service, U. S. Dept. of the Interior. Food Freezing, *1,* No. 2: 48. Dec., 1945.

Fish is one of the most perishable of all commonly used food products, being subject to one or all of several types of deterioration when being frozen and stored in a freezer. The common causes of deterioration are (1) loss of flavor or color due to enzymatic or autolytic action within the tissue of the fish flesh; (2) action of bacteria; (3) oxidation of the fat; (4) "rusting" due to oxygen action on the flesh; (5) freezer burn or dehydration. It is recommended that fish be frozen as quickly as possible after being caught and pending preparation for freezing that they be kept packed in ice stored in a refrigerated room at 33°–38° F. This prevents loss of flavor due to melting ice. If a large quantity of fish is to be handled by a locker plant, a separate room for its preparation should be provided in order to protect other foods from fish odor. All forms in which fish are prepared, including fillets, steaks, sections or whole fish, must be very efficiently wrapped in moisture-vapor-proof material and tightly sealed against admission of oxygen. Low-temperature storage is most essential (–10° F.) for long-time storage of frozen fish, in fact a temperature much lower than that at which locker storage rooms are held.

Two tables are included, one being Chemical Composition of Common Varieties of Food Fish and the other, Estimate of the Probable Storage Life of Frozen Fish. The latter table gives the range in months of storage for fish "round or headed and gutted" and in packaged fillets. The storage life of all fish except those of rather oily nature is from two to four months greater in the fillet form than in the round. L.M.D.

96. Refrigerated Trailers Move Frozen Foods. F. M. REID, Chief Engineer, Fruehauf Trailer Company. Food Freezing, *1,* No. 2: 46. Dec., 1945.

The ability of motor transport to make quick deliveries over long distances, and to any point, is a decided advantage in handling frozen foods. Details of operation of the new Trail-Aire Conditioner, manufactured by the Advance Mfg. Inc., are given. The unit is installed in the nose of the trailer and consists of a compressor powered by a 4-cylinder, V-type, air-cooled gasoline engine, equipped with a starter and generator, and an air-circulating blower of 1800 cu. ft. per min. capacity. This unit functions either for cooling the goods space or for heating it if desired. When cooling, the refrigerating system operates conventionally, the condenser being cooled by the circulated cold air. In order to operate as a heating system, the process is reversed, the evaporator functioning as a condenser and the condenser becoming an evaporator. The efficiency of the heating cycle is increased by supplementing it with the waste heat from the unit's gasoline engine.

Aside from a set of hand-operated change-over valves, the unit is automatically controlled from a thermostat for either the cooling or heating cycle.

L.M.D.

97. Correct Installation of New Refrigerating Equipment Can Mean Difference Between High–Low Maintenance Cost. E. I. ALTER, General Electric Co. Food Freezing, *I*, No. 1. Nov., 1945.

The author stresses the importance of layout of equipment in such manner that it may be accessible for easy and efficient servicing, thus cutting down maintenance expense. He then goes into details of installation of Freon systems. All copper tubing should be cleaned thoroughly with carbon tetrachloride or equivalent before it is assembled on the job. After copper tubing is brazed and the job is ready to run, insert auxiliary felt strainers in the suction line at the compressor to catch any foreign material which may have gotten into the piping during the assembling and brazing. After a short period of operating, the felt strainers must be removed.

The best way to obtain a dry system is to evacuate to a 29-inch vacuum or better, with all parts of the system kept above 70° F. for several hours, while the vacuum is being held. Alternative treatment is pulling the best vacuum possible on the system and remove remainder of moisture by operating with silica gel driers in the liquid lines. Change drier cartridges frequently until moisture has disappeared, then valve out the drier from the circuit.

Avoid oil and refrigerant trapping in the evaporator circuit piping. Whenever a compressor is started up after a period of shutdown, oil is usually pumped out of a compressor at a faster rate than it is returned. Trapping of oil in the low side may then prevent quick return of the oil to the compressor which may result in bearings failure. Especially avoid traps formed by a vertical drop from the evaporator followed by a long horizontal run with a short vertical rise to the suction service valve of the compressor. Liquid line solenoid valves in the refrigerant circuit are recommended to minimize drawing slugs of oil and refrigerant into the compressor when it starts up.

Heat interchangers are recommended where the refrigerant temperature in these systems is usually below zero degrees F. or lower. The heat interchanger increases the effective capacity of the system within the refrigerated space by cooling the incoming liquid refrigerant and warming the suction gas. This reduces the problem of frost on the suction line and makes possible less suction line insulation.

Defrosting by means of an automatic brine spray type evaporator is indicated where regular attention of an attendant is not possible.

When a system must be run the year around it is important to have at hand adequate replacement parts and where continuous duty is imperative, provision should be made for standby equipment which can handle the load while major repairs, such as compressor or condenser replacements, are being made. L.M.D.

98. **Tommorrow's Package . . . The story of Aluminum foil.** T. M. Hill, Foil Division, Aluminum Company of America. Food Freezing, *1,* No. 2: 52. Dec., 1945.

The close of the war released aluminum foil once more for civilian use. Long used in the confectionery and processed cheese industries, aluminum foil offers many potentialities as a general food protective wrapper and particularly to the rapidly developing frozen foods industry. General characteristics of aluminum foil are: (1) Protection, (2) Odor Proofness, (3) Gas Proofness, (4) Light Proofness, (5) Glitter, (6) Dead Folding, (7) Dimensional Stability, (8) Aging Characteristics, (9) Insect Proofness. The four thicknesses typical of those used in the packaging industry are given together with the covering area in square inches per pound.

Thickness in inches	*Covering area in sq. in. per lb.*
0.001	10,250
0.0007	14,600
0.0005	20.500
0.00035	29,300

One defect appears in the thinner gages of foil which is that of pin holes which is said to be unavoidable. This permits some gas transfer.

Aluminum foil is very adaptable to being coated with synthetic resins, backed with paper or cloth, and to being coated with heat sealing material to provide nearly hermetic seals. As a liner for cartons it is bound to find great use in frozen food packaging. Large sheets of foil may be used for wrapping large units of either regular or irregular shapes. Because of the rather common use of aluminum cooking utensils, it is pointed out that foil-wrapped foods may be introduced into pot or pan for cooking without removing the wrapper. Also the use of foil wrappers should be satisfactory for pre-cooked frozen foods. L.M.D.

99. **Women Want Informative Labels.** Dorothy B. Marsh, Food Editor, Good Housekeeping Institute. Food Freezing, *I,* No. 1. Nov., 1945.

Though frozen foods have been on the market for twenty years, many women still shy from using them. With an expanding market hoped for in the days to come, many new brands will come into competition with each other. The brands which will ultimately hold their own—such items as quality of product and efficiency of package protection being equal—are those wihch convey adequate information to the housewife (the retail consumer) about proper cooking, number of servings per package (and not skimpy ones either), and number of cupsful. This latter for vegetables in both raw and cooked state because they may be used as recipe ingredients. If the product contains added sugar, state kind and amount so user can allow for it when using the product in a recipe. Also give quantity equivalent in terms of "as purchased" unprocessed market vegetables. In the case of meat, poultry and fish, point out that the product is ready for oven, pot or skillet with no waste. Directions for proper storage of frozen foods should be given also to cover various lengths of time before using. Above all, be sure that the contents of the package lives up to its label—the label being the window of the package. (All this will call for utilizing most of the six surfaces of a rectangular package for printed information and helpful instructions, but it will be good sales aid and bring repeat orders.)

L.M.D.

100. **Oil Problems in Freon Refrigerating Systems.** T. J. AMMEL, Sales Engineer, Nash-Kelvinator Corp., Detroit, Michigan. Refrig. Engin., *50:* 5. Nov., 1945.

Oil problems with self-contained equipment, principally in the smaller unit field are discussed. Because of the ease of miscibility of Freon with oil, the oils used should be of a somewhat higher viscosity generally than those used with ammonia or sulfur dioxide to overcome "thinning" action of the refrigerant. The oil must be heavy enough to provide a good body for lubricating purposes—yet it must remain fluid at temperatures which go down as low as – 40° F. in single stage systems and considerably lower in two and three-stage systems. Today most manufacturers specify an oil of 280 to 300 viscosity for domestic and commercial machines in the smaller sizes. Oils in this viscosity range have a flash point of 350° to 360° F. and a pour test of – 25° F. The pour point is further reduced by admixture of refrigerant.

The methods of compressor lubrication are used in the small compressors, splash system for the open type reciprocating units and forced feed system in the hermetic units.

Pumping of oil by compressors is kept to a minimum or eliminated entirely by the adoption of some or all of the following improvements: (1) Burnishing or superfinishing of cylinder walls and pistons to a clearance of from 0.0003 in. down to 0.0001 in. rendering piston rings unnecessary in fractional horsepower models; (2) Use of special close-grained metals for castings and cylinder walls rather than conventional cast iron; (3) The use of reed type valves, and ring-plate valves on larger compressors; (4) Streamlining of suction and discharge parts and passages; (5) Development of the balanced pressure seal; (6) Adoption of neophrene synthetic rubber compounds for gaskets and shaft seals.

To further facilitate the oil-handling problem, more attention has been paid to low side design to keep vapor velocity correlated with evaporator temperature, the lower the temperature the greater should be the terminal velocity of the vapor: 1000 ft. per min. for high operating temperatures and progressively increase to say 2000 ft. per min. at 0° F. or below. This prevents oil logging. The more oil in a Freon system the less efficient the refrigeration, thus 5% oil in Freon results in 4% reduction of evaporator capacity to 63%. Another accessory is the oil separator which returns oil from the discharge gas to the compressor crankcase through an oil return line but while it is necessary for the open type reciprocating machines, it is not needed with the hermetic systems. Lastly, low temperature applications on the order of – 100° F. have been made possible by the development by the refiners of lubricating oils that show no indication of wax separation over long periods of operation.

L.M.D.

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CONTENTS

Bacteriology
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Cheese
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Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
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ABSTRACTS OF LITERATURE

BOOK REVIEW

101. **Analysis of Foods.** ANDREW L. WINTON AND KATE BARBER WINTON. John Wiley & Sons, New York, 1945.

A compilation of methods for the determination of the constituents of foods in six groups, methods which have been accepted by American National organizations or are standard in other countries. Others in addition are included which have been published in accredited journals but which are either tentative or have not been given that quasi-acceptance, yet are in extensive use. The compilers have been to great trouble to separate method proper from experimental and discussion entanglement in journal articles and putting it in a lucid and usable whole. In this they have succeeded admirably.

Part I. Brief treatment is given to General Microscopic and General Physical Methods. These are then followed by General Chemical Methods, which constitute about half of the content and cover: 1. Organic Elements; 2. Constituent Groups; 3. Water; 4. Protein; 5. Fat; 6. Nitrogen-free extract; 7. Fiber; 8. Ash (2 through 8 the six groups); 9. Alcohols; 10. Vitamins; 11. Natural Colors; 12. Artificial Colors; 13. Chemical Preservatives (10 through 13 are classed as Traces, while 12 and 13 are both classed as Extraneous Constituents).

Part II. Special Methods. This comprises the methods which have in large part become standard analysis procedure for: A. Cereal Foods; B. Fatty Foods; C. Vegetable Foods; D. Fruit Foods; E. Saccharine Foods; F. Alcoholic Beverages; G. Dairy Products; H. Animal Foods; I. Alkaloidal Products; J. Food Flavors; K. Leaven; L. Salt. 208 illustrations, numerous tables, and a liberal use of chemical formulas along with necessary mathematical computation methods. This book can serve to advantage as a time-saving instrument in the food analysis laboratory. L.M.D.

BACTERIOLOGY

102. **An Improved Darkfield Quebec Colony Counter.** O. W. RICHARDS AND P. C. HEIGN, Research Division, American Optical Co., Buffalo, N. Y. Jour. Milk Technol., *8*, No. 5: 253–256. Sept.–Oct., 1945.

The improved colony counter has an annular reflector which gives uniform oblique lighting. The colonies stand out against a darkfield clearly. Colonies can be examined in a colored culture medium by using a colored or light field. The counter has a single or double magnifying glass, designed to hold Jeffers, Stewart, or Wolffhengel guide plates. The instrument is protected from dust, is convenient to manipulate and does not become hot while in operation. H.H.W.

BREEDING

103. Livability and Glycolysis of Bovine Spermatozoa in Yolk-citrate, Incubated Eggs, or Chick Embryo Diluters. G. W. SALISBURY, J. A. ZELAYA, AND N. L. VANDENMARK, Cornell University, Ithaca, N. Y. Jour. Animal Sci., *4*, No. 3: 270–276. Aug., 1945.

Two different experiments were conducted, one with semen collected in August and the other collected in March. Each ejaculate of semen was diluted at the rate of one part semen to four parts of each diluter used. The chick-embryo diluent made from whole-fertile eggs incubated nine to eleven days was only slightly superior to the yolk-citrate in maintaining livability of the spermatozoa during low-temperature storing. The semen samples in the chick-embryo diluent produced slightly more lactic acid during storage than did the same semen samples in the yolk-citrate. This was associated with a loss of glucose and suggests that the chick-embryo material aided in the promotion of glycolysis by the spermatozoa. C.F.H.

BUTTER

104. Use of an Antioxidant in Preventing Hydrolytic Rancidity in Dairy Products. I. A. GOULD, University of Maryland, College Park, Md. Natl. Butter and Cheese Jour., *37*, No. 1: 40. Jan., 1946.

The addition of 0.75 and 1.5% Avenex concentrate to raw cream retarded lipolysis and rancidity in the resulting raw-cream butter. The addition of the same material to raw cream did not appreciably affect the lipolysis which resulted when the cream was subsequently homogenized at low pressure. It is possible that other antioxidants may control rancidity. Those factors affecting rancidity development in normal raw milk do not have the same effect on lipolysis induced by homogenization. W.V.P.

105. The Butter Industry at the Crossroads. RUSSELL FIFER, American Butter Institute, Chicago. Natl. Butter and Cheese Jour., *37*, No. 1: 36. Jan., 1946.

In the year 1945 milk production reached a new high, but butter was the shortest in history. This paradox was caused by military purchases and unfair price restrictions. Removal of these restrictions should bring a normal price relationship with other manufactured dairy products within a reasonable period. Consumer, industrial, and institutional markets must be attained by production of a quality product, and by emphasizing butter's nutritive properties. Surveys by American Dairy Association show that a large majority of consumers prefer butter to other spreads. W.V.P.

CHEESE

106. **A Phosphatase Test for Cheddar Cheese.** G. P. SANDERS AND O. S. SAGER, Division of Dairy Research Laboratories, U. S. Dept. Agr., Washington, D. C. Jour. Milk Technol., *8,* No. 4: 223–226. July–Aug., 1945.

The method is a modification of the phosphatase test used in testing inadequate pasteurization of milk. Complete details of the procedure will be available at a later date. Over 340 samples of Cheddar cheese with complete records of the milk treatment were tested. Cheese made from raw milk or under-pasteurized milk gave a positive phosphatase reading. Cheese from pasteurized milk at 143° F. for 30 minutes or 162° F. for 15 seconds gave values greater than 5 units, regardless of the age of the cheese. The addition of 0.1 per cent of raw milk to pasteurized milk could be detected in the cheese test. One sample of raw-milk cheese after five years and several cheeses held three years all gave a strong phosphatase test. H.H.W.

107. **A Discussion on Standards for Soft and Semi-Soft Cheese.** RAYMOND MIOLLIS, Natural Cheese Co., Maywood, Ill. Natl. Butter and Cheese Jour., *37,* No. 1: 72. Jan., 1946.

Moisture and fat regulations such as govern the Cheddar cheese industry would be unfair to use for controlling the soft cheese industry. Such regulations might discriminate against some types of cheese, might interfere with the development of new types, might introduce complications in controlling imports, might lead to frequent revisions of definitions for many varieties of cheese, and might be extremely laborious to enforce. The Dutch method of branding cheese with the minimum fat per cent in the dry matter would be preferable. W.V.P.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

108 **Sanitary Standards Program of the Evaporated Milk Industry.** E. H. PARFITT, Evaporated Milk Association, Chicago, Ill. Jour. Milk Technol., *8,* No. 2: 108. Mar.–Apr., 1945.

The Evaporated Milk Association has formulated a sanitary standards code, which has been adopted by 96 per cent of the industry.

The program and accomplishments are discussed. L.H.B.

109. **Water Absorption of Plastics Molded from Acylated Casein.** W. G. GORDON, A. E. BROWN, AND C. M. MCGRORY, Eastern Regional Res. Lab., U. S. Dept. Agr., Philadelphia, Pa. Jour. Indus. and Engin. Chem. Indus. Ed., *38,* No. 1: 90–94. Jan., 1946.

Casein plastics constitute only a small percentage of the total production of plastics, one reason being the relatively high water absorption properties of the protein. The modification of casein by acetic, propionic, and n-butyric anhydrides to produce derivatives which can be molded directly into articles having greater resistance to water was studied. Preparation of acylated products containing 3.4 to 10.1% acetyl, 2.7 to 13.4% propionyl, and 7.5 to 14.9% butyryl is discussed. Water absorption tests on disks molded from these materials demonstrated that the affinity of casein for water is curtailed progressively by increasing the number and size of the acyl groups incorporated in the modified protein. This reduction in water uptake is accompanied by a decrease in the strength of the molded articles. B.H.W.

110. "Wonder Food" Made from Casein. ANONYMOUS. Natl. Butter and Cheese Jour., *36,* No. 12: 34., Dec., 1945.

Amino acids prepared by the acid hydrolysis of casein are being distributed in sterile liquid and in powder forms. The product has possibilities in healing of wounds, rejuvenation of starved people and for the feeding of sick people who could not eat otherwise. W.V.P.

DISEASE

111. Mastitis and the Plate Count of Milk. VI. The Contribution of Staphylococcal Mastitis to the Standard Plate Count of Milk, and Some Cultural Characteristics of the Organisms Isolated. H. W. SCELEY, JR., E. O. ANDERSON, AND W. N. PLASTRIDGE, Storrs Agricul. Expt. Sta., Storrs, Conn. Jour. Milk Technol., *8,* No. 5: 259–263. Sept.–Oct., 1945.

Staphylococci were identified in samples of milk drawn aseptically from quarters affected with Staphylococcal mastitis. The numbers of Staphylococcus colonies ranged from 200 to 42,000 per ml.

A total of 289 cultures of staphylococci were isolated from the infected quarters; of these 209 were coagulase-positive or 72 per cent; 156 were hemolytic on blood agar or in blood broth. This constituted 36 per cent of the total staphylococci. Thirty-five of the cultures or 44 per cent produced some pigment ranging from a deep yellow to a white pigment.

No relationship existed between the leucocyte counts and the corresponding plate counts of the same samples. H.H.W.

112. The Therapeutic Effect of Yeast and Pyridoxine on Poikilocytosis in Dairy Cattle. J. T. REID, C. F. HUFFMAN, AND C. W. DUNCAN. Michigan State College, East Lansing, Mich. Jour. Nutr., *30,* No. 6: 413–423. Dec., 1945.

Young calves on semi-restricted ration and cows with large open fistulas of the rumen which showed poikilocytosis were used in this study. Dry brewers yeast, live yeast, or pyridoxine were effective therapeutic agents for the treatment of this condition, whereas the ingestion of nicotinic acid, riboflavin or a mineral mixture containing iron, copper, cobalt, and manganese did not elicit any curative effects on the disease. The occurrence and the degree of severity of poikilocytosis were independent of the hemoglobin content; red cell count, red blood cell volume, and the plasma concentration of calcium, inorganic phosphorus, and magnesium. The hypothesis was advanced that the primary defect responsible for the occurrence of poikilocytosis in dairy animals is due to a lack or an interference with normal ruminal activity. C.F.H.

113. A Review of Bloat in Ruminants. H. H. COLE, C. F. HUFFMAN, M. KLEIBER, T. M. OLSEN, AND A. F. SCHALK. National Res. Council Fourth Report of Committee on Animal Health, National Res. Council, Sub-com. on Prevention of Bloat. Jour. Animal Sci., *4*, No. 3: 183–236. Aug., 1945.

This is a review of the literature pertaining to bloat in the ruminant under the following headings: 1. Seriousness of the bloat problem; 2. Conditions under which bloat occurs; 3. Sources of rumen gases; 4. Factors influencing rate and type of gas formation; 5. Expulsion of gas from the rumen; 6. Experimental production of bloat; 7. Prevention of bloat; 8. Treatment of bloat; 9. Theories of bloat. C.F.H.

FEEDS AND FEEDING

114. Vitamin Requirement in Calves. J. M. LEWIS AND L. T. WILSON, Dept. Pediat., New York Univ. and Walker Gordon Lab., Plainsboro, N. J. Jour. Nutr., *30*, No. 6: 467–475. Dec., 1945.

Holstein calves of either sex, two to nine days of age, were placed on a ration nearly devoid of vitamin A in order to deplete their stores of this vitamin. Upon the cessation of gain in weight, they were divided into six groups of four calves each and placed on the following levels of vitamin A from shark liver oil (50,000 U.S.P. units per gm.): 32, 64, 128, 256, 512, and 1023 U.S.P. units of vitamin A per kg. body weight per day, respectively. The results indicated that the minimum vitamin A requirement for growing calves is 32 U.S.P. units per kg. body weight and that the requirement for maximum growth is 64 U.S.P. units per kg. body weight. The concentration of vitamin A in the blood increased with the vitamin A intake to 512 U.S.P. units when maximum concentrations were obtained. The authors concluded that from the standpoint of both growth and liver storage, the daily intake of vitamin A for young calves should be about 250 U.S.P. units per kg. of body weight. C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

115. The Dietary Importance of the Fatty Acids in Butterfat. ANONYMOUS. Natl. Butter and Cheese Jour., *37,* No. 1: 68. Jan., 1946.

A review of the subject released in "Dairy Council Digests," November, 1945, by National Dairy Council, 111 N. Canal Street, Chicago, Illinois.

W.V.P.

116. Nutritional Studies on Milk Fat. I. The Growth of Young Rats Fed Milk Fat and Certain Synthetic Glycerides as Supplements to a Fat-free Diet. J. L. HENDERSON, E. L. JACK, S. LEPKOVSKY, AND D. F. REID, Division Dairy Ind., Univ. Calif., Davis, and Div. Poultry Husbandry, Univ. Calif., Berkeley, Calif. Jour. Nutr., *30,* No. 3: 169–173. Sept., 1945.

Two trials were made using young rats fed a fat-free diet for the purpose of comparing the following supplements for growth. One group received twenty per cent butterfat as a supplement; a second group, twenty per cent triolein; a third group, twenty per cent trilaurin; and a fourth group, ten per cent each of triolein and trilaurin. The results showed that the rats fed the milk-fat diet and the triolein diet grew faster than those fed the other diets. There was no significant difference between the milk-fat diet and the triolein diet with respect to growth. C.F.H.

117. Nutritional Studies on Milk Fat. II. The Growth of Young Rats Fed Glyceride Fractions Separated from Milk Fat. E. L. JACK, J. L. HENDERSON, D. F. REID, and S. LEPKOVSKY, Div. Dairy Ind., Univ. Calif., Davis, and Div. Poultry Husbandry, Univ. Calif., Berkeley, Calif. Jour. Nutr., *30,* No. 3: 175–181. Sept., 1945.

Ten groups of young rats were used in this study. Five glyceride fractions separated from milk fat by precipitation from a solvent were fed as supplements to a fat-free diet to weanling rats, and the gain in weight measured. The growth responses placed the fractions in three groups. The – 50° C. filtrate was superior to all other diets containing solvent-treated fat; the – 70° C. ppt. and the fat-free were the poorest; the others formed an intermediate group. The diet containing natural fat which had not been solvent-treated gave greater growth than any other diet. The diets containing the solvent-treated milk fats (natural, synthetic and composite) fell in the intermediate group with no significant difference between them. The authors suggested that either the oleic acid content or the total unsaturation might be a factor contributing to differences in growth. The – 53° C. fraction contained more oleic acid than the other fractions. C.F.H.

ICE CREAM

118. **How to Make Ice Cream without Fluid Cream.** C. D. DAHLE, Pennsylvania State College, State College, Pa. Ice Cream Field, *46,* No. 6: 18. Dec., 1945.

It is pointed out that when fresh cream is not available, butter, plastic cream, and frozen cream may be used to supply the necessary fat in ice cream. Brief mention is made of the desirability of carefully selecting these products if good quality ice cream is to be produced. If difficulty occurs in getting the desired overrun in batch freezers when such products are used, the addition of one-half per cent egg yolk to the mix should improve whipping conditions. W.C.C.

119. **The Future of Powdered Mix in Ice Cream.** P. H. TRACY, Dept. of Dairy Husbandry, Univ. of Illinois, Urbana, Ill. Ice Cream Field, *46,* No. 6: 22. Dec., 1945.

Powdered ice cream mix—a development of World War II—will likely find household and commercial uses. It is stated that normally 15–20 per cent of the housewives make their own ice cream. This should serve as a potential market provided that powdered mix will reconstitute and whip readily and have a good flavor. It is predicted that packaged powdered mix will be generally available in grocery stores and that household mechanical refrigerators will likely be equipped with small ice cream whipping units and facilities for faster hardening. W.C.C.

120. **Use of Homogenized Cream in Ice Cream.** ALAN LEIGHTON, Bureau of Dairy Industry, U. S. Dept. Agr., Washington, D. C. Ice Cream Field, *46,* No. 6: 30. Dec., 1945.

Experimental mixes in which all of the ingredients were mixed, then pasteurized, homogenized, cooled, and aged were compared with mixes of the same composition in which the cream was pasteurized and homogenized before adding it to the rest of the ingredients after they had been pasteurized, cooled and aged. The author used as a measure of the whipping properties of the various mixes the maximum overrun obtainable under the experimental freezing conditions.

It is shown that 12 per cent butterfat mixes of satisfactory whipping properties can be made from pasteurized, homogenized and aged cream. If difficulty occurs, it is most apt to be with mixes made from 25 per cent cream, it is stated. Longer aging periods for the mix and the use of (a) alginate, (b) gelatin-monoglyceride, or (c) carboxy methyl cellulose as stabilizer improved the whipping properties of such mixes. W.C.C.

MILK

121. The Methylene-Blue Reduction Test as a Means of Estimating the Bacterial Content of Milk to Determine Its Suitability for Pasteurization or as a Basis for Grading.

C. A. ABELE, Health Dept., Chicago, Ill. Jour. Milk Technol., *8*, No. 2: 67. Mar.–Apr., 1945.

The U.S.P.H.S. Milk Ordinance specifies that raw milk produced for Grade A pasteurized milk must have an average plate count not in excess of 200,000 per ml. or an average methylene-blue reduction time of six or more hours.

Since it was impractical and costly to make plate counts on all raw samples of milk because of the large number of farms from which the Chicago supply is obtained, the methylene-blue reduction test was used. It was found, however, that milk supplies meeting the six-hour average reduction time requirement did not, in Chicago's experience, result in a pasteurized product, which uniformly met the requirement of a plate count of 30,000 or less per ml.

A study was made comparing the reduction times with the plate counts. In this study it would appear to justify the assumption that any milk supply that had a reduction time of less than six hours is likely to have a plate count in excess of 200,000 per ml. However, it also shows that when the test is conducted according to Standard Methods (8th Ed.), that a relatively large percentage of samples having a reduction time of more than six hours will have a plate count over 200,000 per ml. It is obvious that the rising cream sweeps many bacteria up with it, thus extending the reduction time of the defatted portion, which is really the portion that determines the reduction time of the sample.

When tubes were inverted at 30-minute intervals during incubation to redisburse the fat and results compared with same samples when tubes were not inverted, it showed that inverting the tubes gave a higher degree of agreement betwen plate counts and reduction times. It was noted that most of the reduction times were shortened by inverting tubes. L.H.B.

122. Observations Concerning the Methylene-Blue Reduction Test.

C. K. JOHNS, Dominion Dept. of Agr., Ottawa, Canada. Jour. Milk Technol., *8*, No. 2: 80. Mar.–Apr., 1945.

The methylene-blue test was first used largely for the rapid detection of the poorer quality milks, and has been extremely useful in bringing about improvement in the quality of milk. It is not considered reasonably accurate by some investigators, however, when the reduction time exceeds 5½ hours. Therefore, it is not surprising that the reduction time on the better grades of milk have failed to show good correlation with the plate count.

The lack of correlation between plate count and reduction time may be due to a number of factors, namely, the reducing activity varies with the species of bacteria present, and unfortunately, many of the types which are heat resistant have weak reducing powers. The reduction time is influenced by the state of activity of the bacteria. Bacteria which are dormant from prolonged refrigeration will require a longer time to reduce methylene-blue than when in a more active state of growth. The sweeping of bacteria in varying proportions to the surface by the rising butterfat is also a great factor. Inversion of the tube at regular intervals will eliminate this factor. The incubation temperature of 37° C. is not the optimum temperature for many bacteria present in milk.

The plate count should not be considered as an infallible yardstick for measuring quality. It merely indicates the number of colonies which develop on a certain medium at a given temperature for a definite period. When any of these specifications are changed, marked variations in the counts may be obtained. Also, a colony on the plate count may be formed from a single organism or from a clump of 100 or more.

The methylene-blue reduction test has an advantage in the size sample used. On raw milk a dilution of 1:1000 or higher may be used for the plate count. Thus, only one thousandth of a milliliter or less of milk would be used as compared to 10 ml., or 10,000 times as large a quantity for the reduction test.

In view of the changes which have been made in both the methylene-blue test and the plate count since the equivalent values for these two tests were established by the U.S.P.H.S., it is suggested that it would be desirable for this organization to investigate the situation with a view to establishing a new set of equivalent values, if necessary. L.H.B.

123. Practical Applications of Several Coliform Tests to Pasteurized Milk. W. D. Tiedeman and S. E. Smith, State Department of Health, Albany, N. Y. Jour. Milk Technol., *8*, No. 6: 323–330. Nov.–Dec., 1945.

The authors conclude that bottled pasteurized milk is not free from coliform bacteria, particularly during warm weather. The fermentation test is not entirely satisfactory for examining the presence of coliform organisms because the method does not differentiate milk containing 2 or 3 coliforms per ml. as compared to milk with several thousands. A quantitative estimate of these bacteria can be obtained by using desoxycholate or violet red bile culture media.

A temporary year around standard for raw certified milk is less than 10 coliform organisms per ml. It may be possible to set a higher standard after plants have reached a higher sanitary standard. Coliform organisms in 1 ml. of pasteurized milk can be obtained in clean sanitary plants.

H.H.W.

124. Routine Examination of Milk for Added Water. Hermann C. Lythgoe, Director, Div. of Foods and Drugs, Mass. Dept. of Public Health, Boston, Mass. Jour. Milk Technol., *8*, No. 2: 101. Mar.–Apr., 1945.

The following procedure is used in the laboratory: 1. Total solids are determined by evaporation on a steam bath by Leach's method; 2. Fat is determined by the Babcock test.

From these tests, samples suspected of being skimmed or watered are set aside for further tests. Protein determinations are made on samples when skimming is suspected, and when watering is suspected, the following tests are made for the detection of added water: 1. The refractive index of the copper milk serum; 2. The freezing-point test; 3. The ash of the sour milk serum or the ash of the acetic acid milk serum.

The author reports that "the freezing point of milk is more susceptible to changes by the addition of water than are the other constants, but if watering is suspected by this figure, further examinations should be made to confirm this by using other methods of analysis. In all cases, where the figures are not far from normal, samples of known purity are desirable, if not necessary." L.H.B.

125. The Effect of Temperature on Coliform Organisms in Milk and Cream. Elizabeth D. Robinton and Elizabeth F. Geming, Dept. Bacteriology, Smith College, Northampton, Mass. Jour. Milk Technol., *8*, No. 2: 97. Mar.–Apr., 1945.

In tests made in this study, it was found that coliform organisms have a greater ability to multiply in cream than in milk. Cream is apparently a more favorable medium than milk.

Plates incubated at 20° C. for 48 hours showed much higher counts than duplicate plates incubated at 37° C. for 48 hours.

Coliform organisms develop rapidly even at refrigeration temperatures of 8° C.

Preliminary tests indicated that *Aerobactor aerogenes* organisms increased to a greater extent than did *Escherichia coli* organisms. In later tests this did not always hold true. L.H.B.

126. Report of Committee on Dairy Farm Methods. (Internatl. Assoc. Milk Sanit.) Jour. Milk Technol., *8*, No. 2: 95. Mar.–Apr., 1945.

The committee has undertaken certain studies pertaining to the cleaning and sterilizing of milking machines. The following four items were suggested for study:

Polyphosphates vs. Lye Storage.

Chlorine vs. Lye Storage.

Dry Storage vs. Certain Types of Wet Storage.

Wetting Agents vs. Ordinary Cleansers.

Some brief studies were made and reported on Chlorine vs. Lye, which indicated no advantage for either method.

Satisfactory results were reported in another instance regardless whether rubber parts were stored in chlorine, lye, or dry storage. Hot water, chlorine, or lye treatments also gave comparable results.

It was found that the attitude of the person doing the cleaning was important. The results obtained by those with the "just get by" attitude were invariably bad. L.H.B.

127. Coliform Organisms in Dairy Products and Their Control. E. L. Fouts and T. R. Freeman, Dairy Products Laboratory, Fla. Agr. Expt. Sta., Gainesville, Fla. Jour. Milk Technol., *8*, No. 2: 89. Mar.–Apr., 1945.

A brief history of both *Escherichia coli* and *Aerobacter aerogenes* and the possible significance of each is given.

Their presence in raw dairy products is generally accepted, but when good sanitary practices are used, their number will be small.

In well-managed and operated pasteurizing plants, coliform organisms are seldom found in 1-ml. portions of freshly pasteurized milk. When they are, it usually indicates recontamination after pasteurization, or improper pasteurization.

When fresh pasteurized bottle milk shows a positive coliform test, a "line test" should be made to find the source or cause of the contamination.

Thorough washing and sterilization of all equipment is of utmost importance.

Charts are given illustrating in a diagrammatic way the methods used in the laboratory to determine the presence of coliform organisms in dairy products. L.H.B.

128. Efficient Refrigeration in the Dairy Plant. Leon Buehler, Chief Refrigeration Engineer, Creamery Package Manufacturing Co., Chicago, Ill. Jour. Milk Technol., *8*, No. 2: 85. Mar.–Apr., 1945.

Adequate and properly controlled refrigeration is necessary for quality products in the dairy industry. The cost of producing this refrigeration is an important item in production costs.

The factors effecting the energy costs for operating the refrigeration plant are discussed and some simple basic rules for keeping energy requirements to a minimum are given. These rules are:

"1. Operate at lowest practical head pressure.
   a. Provide adequate condensing surface.
   b. Use coldest condensing water available in liberal quantity. Scarcity of water, high cost, or inadequate drainage may neces-

sitate reduced quantity or use of cooling tower, spray pond, or evaporative condenser.
c. Keep condensing surface clean and free from scale.
d. Keep system free of noncondensible gas. (Purge.)

2. Operate at highest possible evaporating temperature.
   a. Provide adequate cooling surface.
   b. Keep cooling surface clean of oil on ammonia side and clean of ice., etc., on other side.
   c. Avoid restrictions in suction line. Back pressure valves may be employed for temperature regulation and are a most effective control means. They do, however, violate this rule for highest efficiency.
   d. Balance compressor capacity closely to the load. (Refer back to conclusion number 5)—To attain highest efficiency with fluctuating loads, the plant must be very flexible. Capacity control means include multiple compressor units, speed adjustment, and control attachments to the compressor such as clearance control, cylinder by-pass, or holding suction valves open. Such attachments have rather large losses so that their use does not give the full saving one could expect from the rise in evaporator temperature. In practice it is not possible to provide enough flexibility for operation at optimum conditions at all times.
   e. Use direct expansion equipment. The use of chilled water or brine entails an extra temperature split and results in lower evaporator temperature.
   f. Where cooling to various temperature levels is required (example milk cooling and ice cream freezing), provide compressors for each level so as to operate at highest efficiency for each temperature.
   g. Cool product or space to temperature no lower than necessary.
3. Keep refrigeration requirements to a minimum.
   1. Provide adequate insulation.
   2. Cool product as low as possible by regeneration cycle in pasteurizing (incoming cold raw milk cools hot pasteurized milk) or by water before using mechanical refrigeration."

It is stressed that the refrigeration equipment be balanced against the load. Standby equipment is desirable. L.H.B.

129. A Correlation of the Resazurin Grade with the Standard Plate Count of Raw Milk. N. S. Golding and S. J. Gorgensen, Agricultural Experiment Station, State College of Washington, Pullman, Washington. Jour. Milk Technol., *8*, No. 4: 189–195. July–Aug., 1945.

The authors compared the resazurin test (Lovibond comparator at 10-, 30-, and 60-minute intervals) with bacterial count of raw milk as suggested in Standard Methods. The results were summarized as follows: (1) The resazurin test correlated with the standard plate count, even with milk from widely different sources, and (2) The 60-minute incubation period gave the most consistent results. H.H.W.

130. **Clean Up or Close Up!** HARVEY WEAVERS, Wisconsin Department of Agriculture, Madison, Wis. Natl. Butter and Cheese Jour., *37*, No. 1: 42. Jan., 1946.

The demand in the dairy industry has changed from quantity to quality. Standards are being raised under which all foods are produced and marketed. Quality control is made difficult by acceptance of inferior milk, use of improperly cleaned or defective cans, faulty farm and plant sanitation, defective equipment, improper cleaning and sterilization, lack of pest control, inadequate toilet and hand washing facilities, improperly trained help, poor labor supervision, undesirable plant surroundings, and lack of coordination of plant responsibilities and activities. Undesirable conditions in the industry must be cleaned up. Leading plants are maintaining quality by special attention to the quality of milk and cream received. This is accomplished with quality records, field services, rejection of unsuitable delieveries, premium payments for superior quality, and discouragement of marginal producers.

PHYSIOLOGY

131. **The Levels of Ovarian Hormones Required to Induce Heat and Other Reactions in the Ovariectomized Cow.** S. A. ASDELL, J. DEALBA, AND J. S. ROBERTS, Cornell Univ., Ithaca. N. Y. Jour. Animal Sci., *4*, No. 3: 277–284. Aug., 1945.

Grade dairy heifers from two to three years of age were ovariectomized and used for this study. An average dose of 600 rat units of estradiol-benzoate per day for three days was required to bring ovariectomized heifers into heat. The duration of heat was usually less than one day even though the injections were continued. The mean dose of stilbestrol needed to bring the ovariectomized heifer into heat was 0.255 mg. Uterine muscles of heifers estrogenized after ovariectomy had the same reactions as that of heifers in heat. They were inert to pituitrin, epinephrin, lentin, and arecolin. Progesterone in doses of 35 rabbit units or more given for six days produced the same reactions in the uterine muscle as those found in heifers during the diestrum. The average length of the uterine muscle cells was greater in the estrogenized heifers than in the ovariectomized controls. C.F.H.

MISCELLANEOUS

132. **Refrigeration Abstracts.** Amer. Soc. Refrig. Engin. J. MACK TUCKER, University of Tennessee, Editor. Pub. Quarterly. Editorial Office, Ferris Hall, University of Tennessee, Knoxville, Tenn. General Office, 40 West 40th Street, New York 18, N. Y. $7.00 per year.

The first number of Volume 1 published January, 1946.

Refrigeration Abstracts is being inaugurated by the American Society of Refrigerating Engineers to provide the profession and industry with an adequate index to the literature of refrigeration and the allied fields. A staff of 58 abstractors has been chosen on the basis of their specialized knowledge of the subjects which are assigned to them and because of ther ability to prepare impersonal briefs or abstracts rather than critical reviews. The evaluation of the original articles will be left to the users of the journal. The abstracts are covered under the following classes: 1—General; 2—Natural Sciences; 3—Engineering; 4—Engineering Materials; 5—Refrigerating Media; 6—Thermal Insulating Materials; 7—Machinery and Equipment; 8—Foods; 9—Patents. A list of 347 publications is given which the abstracting staff will cover. Two pages of abbreviations and symbols are presented which will be used in the abstracts to conserve space. The final pages are devoted to the author index. It is to be hoped that in future numbers there will be less abstracts that are titles of articles only. The user of an abstracting service should be provided with at least a modicum of digest of the subject matter of an original article if it is deemed worthy of consideration at all. L.M.D.

133. **High Speed Defrosting of Frozen Eggs.** WM. H. CATHCART, Director, National Bakery Div. Lab., Great Atlantic & Pacific Tea Co. Ice Cream Field, *46*, No. 6: 64. Dec., 1945.

Quality as applied to eggs is defined as pureness, freshness, good flavor and high nutritional value. In addition to proper freezing and storage, rapid defrosting is necessary if quality is to be maintained.

Various methods of defrosting eggs now in use by the industry are outlined. The best of these methods requires about 9 hours to thaw a 30-pound tin of frozen eggs and results in about 40 per cent increase in bacteria content and is the only one of these that gives a product that is normal in body and consistency, the author states. An adaptation of electronic equipment devised for several industrial uses not related to food, made possible defrosting 15-hour items in 15 minutes and 4-hour items in 15 seconds, the author claims. Electronic defrosting is so rapid that there is no time for chemical changes to occur during the process, furthermore, it is stated that there is no breakdown in the fiber and structure of the food, and bacterial growth is held to a negligible minimum. W.C.C.

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CONTENTS

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Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
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ABSTRACTS OF LITERATURE

BOOK REVIEW

134. Food Industries Manual.—An Encyclopaedia of Food Manufacture. Fourteenth Edition. T. CROSBIE-WALSH, General Editor. Leonard Hill, Limited, 17 Stratford Place, W.I., London, 1945.

This revised edition of the Food Industries Manual is divided into a number of sections, each of which is compiled by one or more recognized British authorities in the particular field. Of 1062 pages, 294 are devoted to the Dairy Industry. This section is compiled by the well-known dairy scientist, J. G. Davis, of the National Institute for Research in Dairying, Reading, England. Other fields covered in this Manual are Cereals, Canning and Preserving, Meat Products, Pickles and Sauces, Food Dehydration, Storage and Refrigeration, Packing and several others.

The book is unique in that under each section the material is presented alphabetically in encyclopedic form so that the reader may refer to a particular subject directly without the use of the index. In general, subjects are treated briefly.

The Dairy Industry sections is covered quite generally with by far the greatest emphasis being placed on the bacteriological aspects of dairy technology. Methods, media, equipment and procedures for most bacteriological techniques are outlined in detail. Problems with starters, ropy milk. sweet curdling, etc., are examples of commercial problems, which are well covered. Very little detail is given on the commercial manufacture of butter and practically nothing is presented on the subject of ice cream. Cheese, particularly cheddar and the British varieties are covered in some detail and methods of manufacture are given. D.V.J.

BACTERIOLOGY

135. The Bacteria "Count"—An Estimate Capable of Accurate Interpretation. JAMES D. BREW, University of Tennessee, Knoxville, Tenn., and Robert S. Breed, N. Y. State Experiment Station, Geneva, N. Y. Amer. Jour. Public Health, *35*, No. 7: 683. 1945.

In view of uncontrollable variabilities, agar plate and microscopic estimates should be used conservatively. Estimates may be used safely in judging whether a milk is in "acceptable," "satisfactory" or "unsatisfactory" condition but fine distinctions are not jusified. Wherever the agar plate method is used, it should be remembered that many of the high and low temperature organisms that occur so frequently in pasteurized milk and cream may not grow at 37° C. and that some fastidious organisms will not grow well on even the new standard agar. While some dead bacteria may appear in stainable condition in making microscopic estimates, it should be remembered that dead bacteria are just as significant as the living in interpreting the past history of a given sample of milk. No high quality market milk or cream contains an excessive number of bacteria, either living or dead. M.W.Y.

BUTTER

136. Flavor Reversion in Edible Fats. A. E. Bailey. Southern Reg. Res. Lab., New Orleans, La. Oil and Soap, *23*, 55–58. Feb., 1946.

Bailey defines flavor reversion in fats as "the appearance of objectionable flavor from less oxidation than is required to produce true rancidity" (oxidative). Reversion occurs after the absorption of very small amounts of oxygen. Marine oils and vegetable oils such as soybean, linseed and rapeseed oils that contain fatty acids with more than two double bonds are more susceptible to this defect. In unhydrogenated oils a second type of flavor reversion results in fishiness. This is apparently due to a reaction between phospholipides (probably lecithin) and highly unsaturated fatty acids (or their products).

Flavor reversion in hydrogenated oils is only destroyed by prolonged treatment. Evidence recently obtained shows that isolinoleic acid, a product obtained by hydrogenating linolenic acid in the middle or 12:13 double bond, is probably the source of the flavor and odor defect. Present knowledge, however, does not permit a hypothesis being advanced regarding the mechanism of the reaction between oxygen and isolinoleic acid or other materials actually responsible for the flavor and odor. The solution of the problem may be found in improving hydrogenation processes that will avoid the presence of isolinoleic acid. J.L.H.

CHEESE

137. Clarifying Milk for Cheesemaking. E. G. Hood and I. Hlynka, Dept. of Agriculture, Ottawa, Canada. Nat. Butter & Cheese Jour., *37*, No. 1: 38. Jan., 1946; *37*, No. 2: 39. Feb., 1946.

At one of the larger cheese factories in eastern Ontario, cheese was made from clarified and unclarified milk. It was demonstrated that clarification removed sediment. Cheese quality was improved in flavor and texture. Of the cheese made from clarified milk, a total of 98.7% were first grade as compared with 89.3% of cheese made from unclarified milk. Fat losses in the whey were reduced by clarification from 3.67 to 3.01 pounds of fat per 1000 pounds of whey in one series and from 2.60 to 2.47 pounds in another series. The clarified milk developed acid at a faster rate. No adverse effects of clarification were noted. W.V.P.

138. Manufacture of Parmesan Cheese from Raw and Pasteurized Milk.

E. T. BERTONASCO, Stella Cheese Co., Cumberland, Wis. Natl. Butter and Cheese Jour., *37,* No. 2: 34. Feb., 1946.

The Parmesan cheese made in the United States is the type known in Italy as Reggiano Emiliano or Parmigiano and as Reggiano Lodigiano. The Lodigiano has a lower fat content. Milk is standardized to produce a cured cheese with 36% fat in the dry matter and 32% moisture. Pasteurized whey or milk is prepared for starter by inoculating with equal amounts of *Bacterium lactis acidi* Leichmann, and *Streptococcus thermophilus;* incubation is at 122° F. for 16 hours to develop 0.7 to 0.8% acidity. Starter is added at the rate of 2 to 4%. Liquid rennet or powder is used to give coagulation in 15 minutes at 90 to 95° F. The curd is first cut into pieces the size of a hazel nut and finally reduced to the size of rice kernels. When the particles are "the proper size and firmness," the temperature is raised to 122° to 133° F. in 1½ to 2 hours. From cutting to the end of cooking, the acidity increases from 0.02 to 0.03%. When experience indicates the correct matting point, the curd is dipped by slipping a linen curd cloth under the curd mass and hoisting it out of the kettle with block and tackle. The curd is drained briefly, divided, placed in metal hoops, pressed and later held 2 or 3 days in round wooden hoops called "fascere." The cheese, which at 3 days should contain 40 to 42% moisture, is brine salted for 15 to 30 days, and cured at 58 to 60°. It is turned frequently, rubbed with oil and finally vegetable black. The cheese is cured for 16 to 24 months and averages 6.5% yield.

Defects of Parmesan may be acid texture, acid flavor, lack of flavor, weak, lack of granular structure, or gassy texture. Usually these defects are caused by faulty acid development, improper starter management, lack of moisture control, or inferior milk. Pasteurized milk can be used for Parmesan by increasing the amount of starter and the temperatures of setting, cooking, and curing. The cheese is used chiefly in the grated form or may be dried in 6 to 18 hours at 60 to 80° F. Temperatures of 120° F. or more have been frequently adopted. The dehydrated cheese is sometimes blended with other cheese or dry skim milk or both to alter its flavor or keeping qualities. W.V.P.

CHEMISTRY

139. Oxygen Absorption of Methyl Esters of Fat Acids and the Effect of Antioxidants.

A. J. STIRTON, J. TURER, and R. W. RIEMENSCHNEIDER. Eastern Regional Res. Lab., Philadelphia, Pa. Oil and Soap, *22,* 81–83. April, 1945.

The Barcroft-Warburg apparatus was used to measure the oxygen absorption of methyl esters of fat acids at 100° C. The methyl esters of linolenic, linoleic, oleic and stearic acids and the distilled methyl esters of lard as well as mixtures of the four individual esters were studied. The mixtures absorbed oxygen at a rate that could be approximately predicted from the rate of oxygen absorption of each component acid and the percentage of each present.

The influence of certain antioxidants on the rate of oxygen absorption was also determined. The most effective antioxidants studied were combinations of citric acid with nordihydroguaiaretic acid and with propyl gallate. Citric acid also showed synergistic action with the other antioxidants studied. J.L.H.

140. Purification of Lactic Acid. Production of Methyl Lactate from Aqueous Solutions of Crude Acid. E. W. FILACHIONE and C. H. FISHER. Eastern Regional Res. Lab., U. S. Dept. Agr., Philadelphia 18, Pa. Jour. Ind. Eng. Chem., Ind. Ed., *38*, No. 2: 228–232. Feb., 1946.

A new method for purifying lactic acid or preparing methyl lactate from crude aqueous lactic acid is described. Methanol vapor is passed through aqueous lactic acid, the effluent vapors are condensed and either methyl lactate is recovered by distillation or purified lactic acid is obtained by hydrolysis of the condensate. Other esters of lactic acid can be prepared similarly with appropriate alcohols. The process can be used with crude unfiltered fermentation liquors. The effect of variables on the volatization of lactic acid with methanol vapor was studied. B.H.W.

DISEASE

141. Differential Bactericidal Activity of Bovine Serum Toward Strains of Brucella Abortus of High and Low Virulence. M. R. IRWIN and B. A. BEACH. Jour. Agr. Res., *72*, No. 2: 83. Jan., 1946.

The bacterial action of the serum from both normal and vaccinated cattle has been shown to depend on the combined activity of antibody and complement. The serum of normal animals usually has an appreciable bactericidal activity at dilutions of 1:40 or 1:80, some individuals at 1:160, rarely higher. The serum of animals vaccinated as adults, or nearly so, showed a definite antibacterial activity to *Brucella Abortus* at dilutions of 1:1280 and even at 1:10, 240 in some individuals. The serum of these individuals showed partial agglutinating reactions no higher than 1:100, but with that from one cow at 1:200. In the majority of the tests, undiluted or diluted serum destroyed more organisms of strain 19 than the more virulent strains. These tests show that even in the almost complete absence of agglutinating antibodies, the serum of a vaccinated animal may exert antibacterial activity against *Brucella Abortus* greater than that of serum of normal animals.

These results substantiate a previous report on a parallel finding in the serum of animals once infected, but with a titer of agglutinating antibodies no higher than in normal cattle. H.P.

142. Outbreak of Infectious Hepatitis, Apparently Milk-borne. W. J. Murphy and L. M. Petrie, Georgia Department of Public Health, Atlanta, Ga., and Samuel D. Work, Jr., Forsyth, Ga. Amer. Jour. Public Health, *36,* No. 2: 169. 1946.

The purpose of this report is to present epidemiological evidence that the icterogenic agent of infectious hepatitis may be transmitted by milk. In 1945, a small outbreak of 10 cases occurred in Forsyth, Georgia. All of the patients drank milk supplied by a single dairy. Circumstantial evidence points definitely toward a single milk supply as the source of the outbreak. M.W.Y.

ICE CREAM

143. Commercial Ice Cream Making. Chester D. Dahle, Pennsylvania State College. Circular 277. 30 pages. 1945.

This circular presents a brief but rather complete description of practical commercial ice cream making. The calculation of mixes is covered thoroughly with many type formulas presented as examples.

All phases of ice cream processing are described and definite recommendations and suggestions are made regarding temperatures of pasteurization and storage, pressures for homogenization, etc. Acidity and its standardization is covered in detail with recommendations made regarding the use of various neutralizers.

The freezing process together with the practical problems of overrun control are discussed. The question of hardening and distribution procedures are considered and helpful suggestions made regarding proper handling.

The matter of ingredients for ice cream making is briefly, but well covered. Sugars, stabilizers, flavors and egg products are discussed and recommendations made regarding amounts to use. Suggested formulas for sherbets, ices, frosted malteds and custards are also given. D.V.J.

PHYSIOLOGY

144. Effect of Ambient Air Temperature and of Hand Temperature on Blood Flow in Hands. C. R. Spealman, Naval Medical Research Institute, Bethesda, Maryland. Amer. Jour. Physiol., *145,* 2: 128–222. Dec., 1945.

At any given hand temperature, blood flow was greater the warmer the body. This effect was most pronounced in moderately cold hands (ca. 15° C.) where blood flow in the case of the uncomfortably warm environment was about twenty times that in the uncomfortably cold environment, and least pronounced in very cold hands (ca. 10° C.) where the difference was only about threefold. (The reviewer raises the question whether there is a similar relationship between the blood flow in the cow's body and the udder.) D.E.

145. Consideration of the Mechanism of Neutralization of Endogenous Gonadotrophic Hormone of the Rat by Antigonadotrophic Serum. HERBERT S. KUPPERMAN and ROLAND K. MEYER, Dept. of Zoology, Univ. of Wisconsin, Madison. Amer. Jour. Physiol., *145*, 2: 181–185. Dec., 1945.

The authors point out that the physiological action of antigonadotrophic sera upon the hypophysis and gonads of normal animals produces simultaneously in a single animal both the effects of castration and hypophysectomy. By injection of parabiotic triplet rate they conclude that antigonadotrophic sera can inhibit the endogenous gonad-stimulating hormone in the blood stream after its release from the pitiutary gland. D.E.

146. The Influence of Sodium Lauryl Sulfate on the Biologic Response to the Gonadotrophins and to Insulin. FRITZ BISCHOFF, Santa Barbara Cottage Hospital Research Institute, Santa Barbara, Calif. Amer. Jour. Physiol., *145*, 2: 123–129. Dec., 1945.

The subcutaneous injection of sodium lauryl sulfate produces an edematous area which may be effectively used in delaying resorption of the gonadotropins, thus augmenting the effect of the sheep pituitary and chorionic gonadotropins. D.E.

MISCELLANEOUS

147. Soybean or Vegetable Milk. A Resume and Bibliography. A. K. SMITH, and A. C. BECKEL. Oil and Protein Division, Northern Regional Res. Lab., Peoria, Ill. Chem. and Eng. News, *24*, No. 1: 54–56. Jan., 1946.

A short discussion of soybean milk, its preparation, characteristics and composition as compared with cow's milk is presented. The bibliography includes 124 references. B.H.W.

148. Standards for Measuring Creamery Efficiency. PROF. L. C. THOMSEN, University of Wisconsin, Madison. Natl. Butter & Cheese Jour., *37*, No. 2: 36. Feb., 1946.

Efficiency in a creamery is the effective operation of that business to produce the desired results with a minimum waste of effort. This efficiency cannot be measured simply by butter "overruns." It can only be attained by study of efficient plants, labor costs, composition control, manufacturing losses, utilization of by-products, equipment, cost accounts and market outlets. W.V.P.

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CONTENTS

Bacteriology
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Cheese
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Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

149. **Piping Handbook, Fourth Edition.** SABIN CROCKER, M.E., Senior Engineer, Engineering Division, Detroit Edison Co. McGraw-Hill Book Company, Inc., New York. 1945.

The author, a member of the American Society of Mechanical Engineers, and of several testing and standardization organizations concerned with piping, first published this handbook in 1930. The work was undertaken because of the growing need for an authoritative reference source in the highly specialized field of piping systems in connection with modern power plants, distribution systems, and in industrial operations. It is the endeavor of the author to make this handbook self-contained, insofar as practicable, for the benefit of those who have to deal with design problems without ready access to a reference library.

In the Fourth Edition, the scope of Piping Handbook has been extended to include chapters on "Gas Piping," "Refrigeration Piping," "Hydraulic Power Transmission Piping," and "Corrosion," which also covers the subject of protective coatings. Other chapters have been augmented and still others revamped. In much of this preparation some forty-odd collaborators, experts in the various fields of piping applications involved, have assisted the author in the chapter content. Much fundamental material has been incorporated for mathematical solution of fluid flow, together with graphic methods. Material of this sort which is too cumbersome for handbook inclusion is referred to original source. The 1350 pages contain many tables, charts, diagrams, drawings and a few photographic reproductions of special equipment. The chapter listing furnishes an insight of subject matter.

I. Definitions, Formulas, and Tables; II. Fluids—Properties of Fluids; III. Metallurgy of Piping Materials; IV. Pipes, Valves, and Fittings; V. Heat Insulation; VI. Hangers and Supports; VII. Expansion and Flexibility; VIII. Steam Power—Plant Piping; IX. Building Heating Systems; X. Plumbing Systems; XI. Underground Steam Piping; XII. Water-Supply Piping; XIII. Fire-Protection Piping; XIV. Oil Piping; XV. Gas Piping; XVI. Refrigeration Piping; XVII. Corrosion; XVIII. Hydraulic Power Transmission Piping. L.M.D.

150. **Industrial Oil and Fat Products.** ALTON E. BAILEY, Senior Chemical Technologist, Southern Regional Research Laboratory, U. S. Dept. of Agr., New Orleans, Louisiana. Inter-science Publishers, Inc., New York, N. Y. 1945.

A notable contribution to the field of oil and fat technology, containing much material published for the first time, suitable as a text or a reference and even a handbook on the subject. The book is divided into four sections: A. The Nature of Fats and Oils. In this section the main subjects are, I. The Structure and Composition of Fats and Oils, II. Reactions of Fats and Fatty Acids, III. Physical Properties of Fats and Fatty Acids, IV. Rôle of Fats in the Diet of Man. B. Raw Materials for Oil and Fat Products, V. Sources, Utilization, and Classification of Oils and Fats, VI. Production and Consumption of Primary Fats and Oils, VII. Composition and Characteristics of the Individual Fats and Oils. C. Industrial Utilization of Fats and Oils, VIII. Cooking and Salad Oils; Salad Dressings, IX. Plastic Shortening Agents, X. Butter and Margarine, XI. Bakery Products and Confections, XII. Soap and the Other Surface-Active Materials, XIII. Paints, Varnishes, and Related Products, XIV. Miscellaneous Oil and Fat Products. D. Unit Processes in Oil and Fat Technology, XV. Extraction of Fats and Oils, XVI. Refining and Bleaching, XVII. Deodorization, XVIII. Hydrogenation, XIX. Soapmaking, XX. Fractionation of Fats and Fatty Acids, XXI. Flat Splitting, Esterification, and Interesterification, XXII. Polymerization, and Emulsification. 710 pages, 921 footnote references, 163 tables, and 111 figures comprising graphs, line drawings, flow diagrams and photographic reproductions. L.M.D.

CONCENTRATED AND DRIED MILK: BY-PRODUCTS

151. **Fat Problems in Dairy Products.** Lt. Robert J. Remaley, QMC Laboratory, Chicago, Ill. Natl. Butter & Cheese Jour., *37*, No. 3: 36. March, 1946.

Examination of dry whole milk showed that oxidized flavor may be largely overcome by limiting oxygen to 3% of the gases in the container and by limiting copper to 1.5 ppm. and iron to 10 ppm. Shorter time and lower temperatures of preheating eliminated heated flavor and inhibited stale flavor. Oxidized effects were increased by such reductions. Trials with several antioxidants indicated no significant advantages over gas packaging.

Dry ice cream mix presents problems of quality preservation similar to those of dry whole milk; its keeping properties are not as good.

Good butter oil can be made from butter containing high free fatty acids, or protein decomposition or any physical defect; it cannot be made if the butter is tallowy, metallic, fishy or excessively oily. High heat applied before centrifuging butter or cream improves keeping properties of the oil; extreme heating (240° F.) after centrifuging causes oxidation. Recombined milk made with butter, butter oil or vegetable oil may be more satisfactory than reconstituted dry whole milk. W.V.P.

ICE CREAM

152. Frozen Fruit Purees in Ice Cream. DONALD K. TRESSLER. Ice Cream Field *47*, No. L: 32, 60. Jan., 1945.

Various products in which purees can be used are listed and it is stated that crushed and pureed fruits hold their flavor and color better than fruits not crushed before freezing. The addition of sugar helps to preserve the flavor and color as well as retard oxidation and hence for many purposes is desirable. Protective colloids, such as pectin or gelatin are sometimes beneficial as is the case with "Velva Fruit" and products used in ribbon ice cream. Deaeration of purees is advisable before freezing.

The following formula and procedure is recommended for making pectinized puree from Elberta or other varieties of peaches which brown easily.

Ground peaches 100 lbs., granulated sugar 50 lbs., enzyme converted corn sirup (43° Bé.) 60 lbs., pectin 2.5 lbs., citric acid 1.25 lbs., and water 20 lbs. Stir pectin into 15 lbs. of the corn sirup previously heated to 160° F., then stir this suspension into boiling water. Keep the mixture hot until a smooth sirup results and then add balance of enzyme-converted corn sirup. Peel peaches by scalding in boiling water, steaming for about a minute or dipping in hot lye solution. Sprinkle halved peaches with sugar and citric acid solution before grinding and as fruit comes from grinder, stir immediately into pectin-corn sirup solution maintained at 175° F. Then cool and freeze.

It is stated that the Hale Haven, South Haven, Veefreeze, and J. H. Hale varieties of peaches give especially good pectinized purees and that the following varieties give good purees; Elberta, Ideal, Massasort, Marigold, Vedette, Veteran, Viceroy, Eclipse, Oriole, Rio Oso Gem and Sunbeam. The author states "Pectinized nectarine and apricot purees may be prepared according to the procedure described for peaches. Mixture of peach and nectarine purees and peach and apricot purees are even more inviting than the straight product."

Recipes are also given for pectinized purees of raspberry, boysenberry, loganberry, youngberry, blackberry and blueberry and it is stated that pineapple, grape, plum, cherry and orange marmalade make excellent purees for use in ribbon ice cream.

Directions for making Velva Fruit are also given. W.C.C.

153. Will Vitamined Ice Cream Be "Atomic Bomb" to the Industry? GEORGE BRICKER. Ice Cream Field, *47*, No. 1: 14, 46. Jan., 1946.

It is the author's opinion that vitamined ice cream will rapidly spread until it eventually becomes a standard of quality throughout the nation. He states that there is no great expense or difficulty in adding vitamins to ice cream mix. A listing of the vitamin content of Ardens' Ice Cream is taken from their label. C. W. Crawford, U. S. Food and Drug Adm., is quoted as stating that under present Federal regulations vitamins may be added to ice cream if the product is properly labeled.

It is stated that there is a sharp division of opinion regarding the use of vitamins in ice cream. Fred Yoars, President, National Association of Retail Ice Cream Manufacturers, is quoted as saying that "Enriching ice cream is much simpler and far more practical than it sounds. Alert and progressive ice cream manufacturers can and will capitalize on it to the benefit of the public and themselves alike."

C. W. England, Director of Research, Highs Dairy Products, Washington, D. C., is quoted as follows: "Vitamins are now being added to bread, candy, and various other foods, and we might as well be realistic about the future of vitamins in ice cream."

P. H. Tracy, University of Illinois, stresses ice cream quality and advocates new products, particularly new sweetening agents and vitamins in producing ice cream. A. D. Burke, Dairy Dept., Alabama Polytechnic Institute, sees no objection to added vitamins but warns against over enthusiasm on the use of vitamins.

Strong opposition to adding vitamins to ice cream comes from Harold Gasser, Bard's Dairy Stores Co., Pittsburgh, who states "To add 'medicine' artificially—is too much."

G. Vergil Rector, Fairmont Creamery Company, Omaha, Neb., is not in favor of adding vitamins to ice cream. He states "Plenty has been said and is being said today about ice cream as a food without having to lay undue emphasis on reinforced vitamins."

Frank Leggett, Barber Brothers and Leggett, Evanston, Ill., concurs with the opposition and states "Our contention is that the consumer values ice cream according to its quality as manifest to him in appearance, body and flavor."

C. D. Dahle, Dairy Manufacturing Dept., Pennsylvania State College, is not enthusiastic about the addition of vitamins to ice cream. He states, "A good diet will supply anyone their vitamin needs. Those who are not able to get adequate diets probably are not the ones who will buy ice cream in the first place, and will not buy it in the second place for its vitamin content." W.C.C.

154. **Low Temperatures on the Highways.** M. V. STAGG, Williams Oil-O-Matic Division, Eureka Williams Corp., Bloomington, Illinois. Refrig. Engin., *51,* No. 3: 231. March, 1946.

Truck refrigeration fills in that all-important gap between production or processing of perishables and their arrival at retail outlets. Desirable features of truck refrigeration include low weight, dependability, easy accessibility for adjustment and maintenance, and compactness of unit for minimum loss of pay load space. Factors determining the type of truck refrigeration requirements cover a wide range, some of the most important being, distance of haul, temperature needs varying with product handled, whether or not the haul will be a long-sealed up run or broken by frequent retail stops. Out of the widely varying requirements of the truck refrigeration field, certain systems have evolved. These include: 1. Ice bunker system; 2. Circulating brine system; 3. Dry ice system; 4. Gasoline-powered direct cooling system (with fin plate or blower coils); 5. Truck engine-powered system in two types: direct mechanical connections, and truck-generated electric power used to drive refrigeration equipment by electric motor; 6. Eutectic (synthetic brine) or holdover plates, commonly found in two types of installations: (a) electric-driven condensing unit for plug-in at garage, and (b) garage-mounted condensing unit with make-and-break refrigerant lines. The author feels that the ice bunker system, the circulating brine system, and the dry ice system are identified with the past of truck refrigeration. Ice and dry ice are expensive because of their depletion and brine because of its destructive action. Mechanical refrigeration allows for temperature flexibility and will meet the demands for low-temperature long hauls of frozen foods. Insulation should be employed generously, especially for truck bodies to be used for low-temperature transportation. Interior body design must allow for unrestricted air circulation. All refrigeration piping and cooling units should be fully protected against damage from loading and unloading and from travel vibration. Compressor design must provide for ruggedness and dependability under all factors of highway travel, and especially have a positive lubricating system, preferably of pressure type. L.M.D.

155. Ascorbic Acid Improved Frozen Peach Pack. J. G. Woodroof, R. C. Cecil, Ida Atkinson, and Ethyl Shelor. Food Freezing, *1*, No. 4: 123. Feb., 1946.

The authors studied five varieties of peaches using ascorbic acid to prevent browning of the cut fruit in its preparation for packaging. Several factors enter the problem, such as, whether the fruit is sliced, halved, or pureed; how the peaches are peeled, sliced, mixed with sugar; method of packaging; and whether or not dextrose is to be used for sweetening. While each variety of peach may require different treatment, the following general procedure is advocated. (1) Speed in handling from field box to final container for prepared fruit, (2) Each slice should be coated with sugar or syrup and packed "solid" with no air cavities in the containers, (3) Peeled peaches should be dipped in acid both to neutralize traces of lye and form an acid medium for peach slices, (4) 0.05 per cent ascorbic acid plus 0.10 per cent citric acid to retain color of the fruit, even bleaching it. Low acid varieties of peaches are greatly improved in flavor by the use of either citric or phosphoric acid, especially when used in ice cream. The ascorbic acid prevents oxidation of the thawed fruit besides protecting it in freezer storage. L.M.D.

MILK

156. **Three-Day-a-Week Delivery.** T. Kline Hamilton, Diamond Milk Products Inc., Columbus, Ohio. Internatl. Assoc. Milk Dealers Bul., *38,* No. 3: 71–75. Feb., 1946.

Three-day-a-week delivery, started in Columbus in October, 1943, has the following benefits: 1. All the proven benefits of EOD and daylight delivery are retained; 2. Housewife likes the service; 3. No Sunday work and six-day plant operation eliminates plant relief system; 4. Fifty-two less delivery days; 5. No Sunday work builds employee and consumer goodwill; 6. Through care delivery loads have been leveled over the week compared with other systems as shown in average daily delivery for the week. The last column is the average of corresponding days on each route.

	Seven-day delivery 1940	*Six-day delivery 1941*	*EOD delivery 1942*	*Three-day delivery 1943–1945*	
Monday	96%	121%	99%	102%	99%
Tuesday	96%	102%	94%	96%	
Wednesday	100%	100%	97%	96%	95%
Thursday	99%	99%	99%	94%	
Friday	97%	99%	101%	107%	
Saturday	99%	179%	107%	105%	106%
Sunday	113%	0%	103%	0%	
Average	100%	100%	100%	100%	

7. The square bottle has saved space and keeping quality has been no problem—consumption is increased; 8. This improved delivery system promises to help keep delivery costs in bounds and at the same time give the customer good service, the farmer a good market, and labor a high level of employment and good wages. E.F.G.

157. **Whiting Finds "Homogenized Vitamin D Milk" Big Sales Success.** Robert A. Burns, Whiting Milk Co., Boston Mass. Internatl. Assoc. Milk Dealers Bul., *38,* No. 3: 86–92. Feb., 1946.

Starting with retail routes and later extending the product to wholesale routes, this concern has developed homogenized Vitamin D milk into a substantial part of its sale volume. Sample advertising copy is shown. E.F.G.

158. **How to Hold or Improve Volume on Premium Milk When Wartime Sellers Market Fades.** Anthony Hebner, Adohr Milk Farms, Los Angeles, Calif. Internatl. Assoc. Milk Dealers Bul., *38,* No. 3: 82–85. Feb., 1946.

The first requisite is to have a milk well worth the premium. A wellschooled sales organization and aggressive advertising can sell such milk. Approximately half the volume of this concern over the years has been premium milk. Customers can be more easily shifted to such milks on a falling market and seldom change back. E.F.G.

159. **Three-Day-a-Week Delivery.** ARIEL C. MERRILL, Clover Leaf Dairy, Salt Lake City, Utah. Internatl. Assoc. Milk Dealers Bul., *38,* No. 3: 76–81. Feb., 1946.

Three-day-a-week delivery, used in Salt Lake City for three years, is probably the most efficient delivery system tried to date. Our experience indicates that the milk retains its fresh, sweet flavor satisfactorily. Consumer acceptance is as good as EOD delivery. All drivers have Sunday off and no relief drivers are necessary. A calendar with alternate bold-faced and light-faced type is suggested as an aid to the housewife in keeping her delivery days straight. The cost of delivery has been 0.3 cents less per unit with the three-day-a-week plan than with the nearest in economy, the six-day-a-week plan. However, many things will influence the cost in a particular situation and the ability of a distributor to adopt and maintain the three-day-a-week plan. E.F.G.

MISCELLANEOUS

160. **Tomorrow's Packages**—regenerated cellulose film. D. E. DREW, Supervisor, Packaging Engineering, Section Cellophane Division E. I. duPont de Nemours & Co., Inc., Wilmington, Delaware. Food Freezing, *1,* No. 3: 101. Jan., 1946.

Third in a series of technical discussions of a packaging series. The basic properties of transparent regenerated cellulose film are listed and each discussed briefly. Properties which render cellophane an indispensable adjunct to quick-frozen foods are: Moisture Proofness, Airproofness, Strength, Flexibility, Liquid Resistance, Grease Resistance, Odor Resistance, Temperature Stability, Sealing, Printability, Coverage, and Transparency. Available in (1) Sheets of printed or unprinted cellophane for direct wraps; (2) Rolls for automatic wrapping of cartons, trays, or directly on the product; (3) Sheets, spot-glued or laminated to cartons for inner protective layer or as an outside covering; (4) Bags of all sizes, from small units for home consumption to large bags for 40-lb. bulk packs. Bags may be laminated with paper, foils, carton stock or with other types of plastic films. L.M.D.

161. **Notes on Cooling Towers and Evaporative Condensors.** S. I. ROTTMAYER, SAMUEL R. LEWIS AND ASSOCIATES., Chicago, Illinois. Refrig. Engin., *51,* No. 3: 220. March, 1946.

Water cooling towers are classified in four general groups:

1. The Atmospheric Type, which depends on wind motion for operation.
2. The Natural Draft Type, employing stock or thermal action for production of air movement through the tower.
3. The Mechanical Draft Type, employing forced or induced draft fans for aerating air movement through the tower. The humidfying air washer is a modification of an induced draft cooling tower.
4. The Evaporative Condenser, which is a combination of a refrigerating condenser and a mechanical draft cooling tower.

Each type of cooling tower is described as to constructional and operational details. General information is imparted concerning problems arising from municipal regulations, corrosion, algae growth, and water basins. Cost estimates per ton of refrigerating plant capacity are included.

L.M.D.

162. **Plans and Calculations for Three Typical Locker Plants.** J. A. Smith, Frigidaire Division, General Motors Corp., Dayton, Ohio. Refrig. Engin., *51*, No. 3: 215. March, 1946.

Practical floor layouts are suggested for frozen food locker plants with 120,355 and 1008 lockers. Methods are outlined for figuring refrigeration loads and selecting the proper condensing units and evaporators for them. Plan "A"—120 lockers provides for a small frozen food locker plant as an adjunct to a grocery or meat market and provides for only a limited amount of processing, while the Plans "B" and "C" provide for complete processing, including facilities for fowl, vegetables and fruits. Products loads are calculated on the bases of pounds per locker per day. Plate freezers and forced-air room refrigerating units are employed. L.M.D.

163. **Need of Technological Supervision in the Food Industry. With Special Reference to the Selection, Preparation and Freezing of Fruits and Vegetables. Part II.** H. C. Diehl, Director and Secretary, The Refrigeration Research Foundations, Inc., Berkeley, California. Refrig. Engin., *51*, No. 3: 223. March, 1946.

The first half of the paper was published in the February issue of Refrigerating Engineering. This paper (Part II) discusses the value of pre-cooling harvested produce before it is processed for freezing, and the importance of bacterial control in all parts of the frozen food plant. A detailed list of 45 bibliography items furnishes a valuable source of information for those interested in the technological phases of food freezing. Following the bibliography is the reported discussion of the paper by several experts in the field of food freezing. Emphasis was placed upon the importance of considering all phases of the frozen fruit or vegetable from field production to consumer information on proper utilization of the products, it being conceded that satisfactory refrigeration applications are entirely adequate, but that much research needs to be entered into in order to improve physical and nutritive qualities of the frozen foods. L.M.D.

164.. Research and Quality Control of Pre-cooked Frozen Foods. BARBARA L. HUTCHINGS AND CLIFFORD F. EVERS, The Bird's Eye Snider Laboratories. Refrig. Engin., *51*, No. 1: 26. Jan., 1946.

Pre-cooked frozen foods have provoked great interest in their potentialities in the last several years. Here is a possibility of better food and less waste—but—such products will continue to be expensive food items. The authors emphasize the necessity for the chemist, bacteriologist, cook, mechanical engineer, packing expert and plant manager to work together in solving the many additional problems entering the production picture of pre-cooked frozen foods as contrasted to the freezing of fresh foods.

These problems, in the main, resolve themselves into protective sanitation against contamination from *Staphylococcus aureus* and other food-poisoning bacteria; mold and yeast contamination; enzyme inactivation, especially that of lipase, because of the many fatty foods, sauces and gravies; flavor control, particularly in connection with spices where it has been found that the oils of spices rather than dry spices reduce variability in flavor; the colloidal state of many cooked foods presents the problem of "weeping" control; color; physical attributes of slicing, dicing, stringing, etc.; production equipment design and operating; packaging requirements will be at the maximum of protective qualities; probably requirement of lower storage temperatures for prolonged storage; and consumer education in proper preparation. This latter consideration is one which can do much to promote the consumer acceptance of pre-cooked frozen foods but it calls for painstaking attention on the part of companies entering this field.

L.M.D.

165. Home Freezers During Power Outage. EARL C. MCCRACKEN, LENORE E. SATER, AND KATHERINE B. BAILEY, Bureau of Human Nutrition and Home Economics, U.S.D.A., Washington, D. C. Refrig. Engin., *51*, No. 2: 117. Feb., 1946.

The ability of the home freezer to maintain refrigeration or safe-keeping temperature during an extended period of nonoperation is an important aspect of the frozen food storage problem. It is the concern of the owner-operator, the utility company, and the dealer-service man. The frozen food, if possible, must not warm up to a temperature above that of its crystallization stage which generally lies between 25° and 31° F. in order to retain its palatability which would be greatly reduced by partial decrystallization and subsequent refreezing. In no case should the warm up go beyond 40° F., for the consensus is that no frozen food should be eaten if it has reached 50° F. after having passed through the slow temperature change occurring in a freezer during a power outage. The question resolves itself into how soon it will take the first package in the freezer to reach the limiting temperature. Factors entering into this are efficiency of insulation, size of food load in the freezer, and whether or not the freezer has eutectic plates.

The most vulernable packages are those in the top layer. The shortest time to reach 32° F. with full load was 44 hours and with one-fourth load, 33 hours. The shortest time to reach 40° F. under the same load conditions were 84 and 47 hours, respectively. Packages in the freezing compartment warm more rapidly than those in the storage compartment, because of nearness to the warm machinery. Indications are that more insulation should be supplied in top and lids, and between freezing compartment and compressor.

The authors recommend the development of an alarm device which would warn of temperature reaching the safety limit and which would be independent of the electric power circuit. This would serve to warn of trouble whether it arise from overloading, power failure (blown fuse), equipment failure, or evident general power outage. L.M.D.

166. **The Moisture Losses in Stored Frozen Meats Vary with Packaging Material, Wrapping Method.** NANCY K. MASTERMAN AND KARL WINSOR. Ithaca, N. Y. Food Freezing, *1*, No. 4: 140. Feb., 1946.

Two methods of wrapping meat or variations of these methods are in general use in locker plants, the butcher's wrap and the drugstore or confectioner's wrap. Comparisons were made between both methods of wrapping on pork chops and ground beef, using in one set of observations locker paper and in the other cellophane, both with outer wrap of butcher's paper, frozen and stored for six months. Weighings were made each month in the locker room. In no case was there a loss in weight in the cellophane packages wrapped by either method, while the locker paper packages developed losses in weight varying from 0.88 per cent in chops wrapped with the butcher style to 3.74 per cent in ground beef in the drugstore wrap. In fact, there was a small gain in weight in the cellophane-wrapped packages, indicating a one-way vapor transfer. The cellophane-wrapped meats presented a glazed fresh appearance while the locker-paper wrapped products were unglazed and dehydrated. The greater number of layers of paper in the butcher wrap gave greater protection when locker paper was used than did the one-layer drugstore wrap. The latter wrap results in a flatter package which, when cellophane is used, is most desirable, but the cost is about twice that of locker paper. This leaves it up to the locker plant operator to decide which method will result in the greatest patron satisfaction. L.M.D.

167. The Preservation of Foods by Freezing. CLARENCE BIRDSEYE. Refrig. Engin, *51,* No. 2: Refrig. Engin. Application Data 22. Feb., 1946.

An eight-page résumé of the frozen foods industry touching upon its history, theory of freezing, freezing methods, processing, packaging, storage and transportation, retailing, and the possible developments of frozen food locker plants, together with institutional and farm and home refrigeration.

L.M.D.

168. Tomorrow's Packages. Economical Carton Varieties. THOMAS F. CASS, JR., Sales Research Manager, Container Corp. of America. Food Freezing, *1,* No. 4: 126. Feb., 1946.

The basic requirements for a frozen foods container are: economy, structural strength, merchandising appeal and functional protection to maintain the quality of the packaged food. Two general types of cartons are in use, the tuck end and the top opening. Adaptability to automatic filling is also a consideration. In all cases vapor-moisture proofness must be assured. One method for meeting this need is with laminated boards. Wet-proofness is highly desirable and is now being provided by carton stocks in the manufacture of which special resins are combined with the pulp, and special surface treatments of the board to produce water resistance without using wax surface coatings. Wet-proofness does not provide moisture-vapor transmission protection. This requires thermoplastic laminating or other accessory materials. Descriptions are given of the newest styles of cartons such as the ''Placepak,'' a knocked-down top-opening carton that can be set up with a one hand motion, the cover remaining in upright position; the ''Sealok,'' an end-opening laminated board stock that requires no inner or outer wrap and is sealed after filling; however, it cannot be used for liquid-containing products, unless an inner product container is used; metal-topped and -bottomed fiber cans; a three-piece carton to be set up on production line with special forming and sealing equipment; the Dacca Vapor-Pak, a carton completely processed in the packers plant, which provides a single unit package completely moisture-vapor proofed with thermoplastic inside and out and finally is hermetically sealed; the Vapocan, an adaptation of the Dacca Vapor-Pak, has been developed for home use and locker plants and is a single unit, straight-sided, thermoplastic coated inside and out, provided with a friction plug lid for easy opening and closing. This latter packaging unit is ideal for home freezer use, for it will handle fruits in syrup and fruit juices as well as other frozen products. The top-opening container with accessory protective wrapper must be used for bulky products and large pieces of poultry, meats and fish. The Vapocan adapts itself to serve as a container for liquid type pre-cooked foods frozen in the home.

L.M.D.

169. **Need of Technological Supervision in the Food Industry.** With Special Reference to the Selection, Preparation and Freezing of Fruits and Vegetables. H. C. Diehl, Director and Secretary, the Refrigeration Research Foundation, Inc., Berkeley, California. Refrig. Engin., *51,* No. 2: 123. Feb., 1946.

The necessity for establishing common quality standards for frozen foods becomes more clearly apparent as the industry emerges from wartime conditions to those of a peacetime competitive character. Raw materials must possess the best quality that can be obtained within economic limits, for no frozen food can be better in quality than the raw material of its origin. A plea is made for increased emphasis on basic research into quality items of raw product, such as variety or type, nutritional values, composition, stage of maturity for harvesting, protection of desirable constituents during harvesting and transport to the processing line. Processing, freezing, packaging, storage, distribution and proper preparation by the consumer have received much attention. The author points out that these aspects of the industry have far outstripped the other which requires a much greater devotion of time, basic research being a slower process, and in this case one which has been either slighted or lost to sight, because the rush to develop volume business demands expenditure of funds in promotional channels.

Now is the time to devote a greater portion of the industry's operational capital to the biological properties of its numerous raw materials. The author decries the universal belief that "quick freezing" is in itself the answer to the quality problem of frozen foods, rather it is the industrial tool which renders possible the preservation of quality carefully nurtured in the raw materials. Basic research is needed (already underway at the University of Texas) to establish thermal characteristics of refrigerated foods which will provide more accurate data than at present available to enable engineers to design industrial plants that will be as economical and efficient as possible. Even the interdependence of the geneticist and the engineer is brought to light, the one to develop varieties to possess desired characters as ideally as possible, and the other to accommodate flow sheet and design application to special needs of the raw material. In many cases the refrigeration engineer will find his services extending to the field to provide chilling protection before the food reaches the plant in order to protect quality values. L.M.D.

170. **Milk Freezing for Commercial Use.** John E. Nicholas, Professor of Agricultural Engineering, School of Agriculture, Pennsylvania State College. Food Freezing, *1,* No. 4: 130. Feb., 1946.

In large quantity production of certain types of frozen foods where the individual unit is small, it is more advantageous to freeze in bulk and package afterward, the opposite of the usual locker plant method of packaging first and then freezing. The rapidity of the complete freezing operation, pre-cooling, crystallization, and then sub-cooling is a matter of minutes in air blast bulk freezing as contrasted to hours in pre-packaged one surface plate contact freezing. Two sets of time-temperature graphs are shown to illustrate the fast freezing of bulk commodities, one layer thick on screen trays in 600–800 ft./min. velocity air blast. Complete freezing from an initial temperature of 80° F. to 0° F. was accomplished in from 7 to 11.5 minutes with air blast temperatures varying from –15.9° F. to –13.3° F. The factor of desiccation is variable, being nil or even increased moisture where steam blanching is practiced with carrots, peas, broccoli, etc., to high loss (as much as 25.0 per cent with some leafy vegetables) of moisture. Prepacking and moisture sealing should be followed even in air blast freezing of such products. An outstanding advantage of the bulk freezing is the packing of the product into large bulk containers to be placed in low-temperature storage, pending packaging after the rush of handling peak production is over. This type of operation should provide opportunity for exceptionally large-scale freezing operations. L.M.D.

171. **Tomorrow's Package—rubber hydrochloride film returns.** A. B. Clunan, Manager of Packaging Sales, Pliofilm Dept., The Goodyear Tire and Rubber Company. Food Freezing, *1*, No. 5: 176. March, 1946.

Many commercial films have sacrificed moisture-transmission rate when the materials were processed to remain flexible at low tempratures. A new film, FF Pliofilm, just going into production, has been developed. Research laboratories show that the new film remains flexible at –20° F., and shows an almost zero value for its moisture-transmission rate. It possesses the heat-sealing qualities of the conventional Pliofilm and, in addition can be sealed against a frozen product. Rubber-hydrochloride film is to be supplied in rolls which will be 24 inches wide by 100 feet or 200 feet long. Properties of the new film are: (1) Imperviousness to dilute acids, air, alkali, brine, grease, heat and cold, (2) Dimensionally stable throughout the humidity range, (3) Great tensile strength and resistance to punctures, (4) Non-explosive and not readily flammable, (5) Prints easily, (6) Closes by heat sealing, sewing or adhesives. L.M.D.

ERRATA

Vol. XXIX, No. 4, page A55. Abstract No. 106.

The fifth sentence of this abstract should read: Cheese from milk pasteurized at 143° F. for 30 minutes or 162° F. for 15 seconds did not give values greater than 5 units, regardless of the age of the cheese.

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CONTENTS

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Cheese
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Diseases
Feeds and feeding
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Herd management
Ice cream
Milk
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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

172. Soap in Industry. Georgia Leffingwell and Milton Lesser. Chemical Publishing Co., Inc., Brooklyn, New York. 1946.

In 181 pages and 23 short chapters the authors have presented the many applications of soaps in a number of industries and their uses in several specific products. Two chapters of special interest to the dairy industry are: Chapter I, Animal Husbandry, and Chapter IX, Milk Production. Several formulas are presented in Chapter I covering the use of soap in compounding, cleaning, and disinfecting preparations for livestock equipment sanitation, wound treatment, external parasite control, insect pest repellents, and liniments for treating lameness of horses. The chapter covering use of soap in milk production offers nothing but admonition to use soap for cleaning walls and floors, and general instruction on plant equipment clean up using hot alkali washing solution. It is pointed out that cleanliness of uniforms or work clothes as well as of personnel is an important factor in milk plant appearance. Each chapter carries a bibliography which should prove useful. L.M.D.

173. Glycerin—Its Industrial and Commercial Applications. Georgia Leffingwell and Milton Lesser. Chemical Publishing Co., Inc., Brooklyn, New York. 1945.

The authors present a brief history of glycerin from the year of its discovery in 1779 by Scheele up to the present day, pointing out its many industrial uses and touching briefly on its sources and methods of recovery by the saponification of fats. Then follow twenty-seven chapters of specific industrial applications among which many are of interest directly to the dairy industry, such as—Cleaners and Polishes, Lubricants, Packaging Materials, Plastics, Paints and Protective Coatings, Agriculture, Beverages, Foods, and Veterinary Medicine. In the chapter on Foods, items touched upon are: confectionery, sweetening agent, flavoring preparations, vanilla and other extracts, dairy products, etc. Specific reference is made to the use of glycerin in dairy manufactures. Chapter XXVIII, credited to H. Bennett, takes up Glycerin Derivations known as the non-ionic surface active agents and made up of three classes, non-dispersible, dispersible, and water-soluble. Their chemical formulas, chemical and physical properties are given in detail. The last chapter, XXIX, presents a list of 1583 processes and compositions in which glycerin is an ingredient. Throughout the chapters are numerous formulas in which glycerin is an important constituent, and each chapter, except the one on Agriculture, is supplemented with a bibliography reference. A short appendix contains a table and charts of glycerin physical properties. L.M.D.

BACTERIOLOGY

174. The Formation of Carbon Dioxide by Lactic Acid Bacteria and *Bacillus Licheniformis* and a Cultural Method of Detecting the Process.

T. GIBSON AND Y. ABDEL-MALEK, Bacteriology Department, College of Agriculture, Edinburgh, Scotland, Jour. Dairy Res., *14*, 1 & 2: 35–44. 1945.

The amount of CO_2 formed during the fermentation of sugars by the hetero-fermentative lactic acid bacteria was found to depend on several factors: (1) The sugar fermented. Gas is produced more readily from glucose than from lactose, even in the case of active lactose fermenters. (2) The reaction and the buffering capacity of the medium. The optimum initial reaction of the medium is approximately pH 7 and the greater the buffering capacity the more active is the gas production. (3) Factors which occur in plant juices and yeast. The inclusion in the medium of yeast infusion, yeast autolysate or tomato juice had a markedly stimulative effect on the production of CO_2 from sugars. These factors apparently have a specific activating effect on the mechanism of the fermentation. (4) The concentration of sugar, the optimum being about 5%. The formation of carbon dioxide by *Bacillus licheniformis* was found to be influenced in a similar way, but in this case the optimum initial reaction of the medium is in the region of pH 8–9 and the factors of (3) have little effect.

A cultural test was described in which these organisms produce visible gas, thus differentiating them from other lactic acid bacteria or spore-forming bacilli. The media used are 5% glucose in nutrient gelatin or in a mixture of four parts milk and one part nutrient agar, with the addition where necessary of yeast-extract and a plant juice. The cultures are sealed with a layer of solid agar before incubation. S.T.C.

175. The Effect of Acidity on the Production of Diacetyl by Betacocci in Milk.

G. A. COX, Dairy Research Institute (N. Z.), Palmerston North, Nw Zealand. Jour. Dairy Res., *14*, 1 & 2: 28–35. 1945.

The diacetyl production of betacocci isolated from starters, cheese and rye-grass was investigated at pH values ranging from 5.5 to 4.4. The betacocci produced diacetyl and subsequently partially or wholly destroyed it. The rate of growth of the organisms was progressively slower the lower the pH, but at least as much diacetyl was eventually formed at low pH values as at high pH values. This is taken to indicate that there was an alteration in the metabolism of the organisms, the diacetyl-producing power per unit cell increasing with decreasing pH. S.T.C.

BUTTER

176. Cooling Cream for Churning. S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 7, No. 9: 278. Sept., 1945.

Cream for churning can be cooled over a surface cooler to churning temperature, according to the author, during all seasons but a few months of winter.

Due to the harder fats in winter, cream cooled by surface cooler should be cooled to about 80 or 90° F. only. The rest of the cooling should be done in a vat to protect the resulting butter from becoming sticky or leaky and to make it more spreadable.

The author points out that cooling cream is only one factor influencing the body quality of butter. P.S.L.

177. New Concept About Butter Revealed. DR. W. R. BLOOR, Univ. of Rochester, Rochester, N. Y. Amer. Butter Rev., 7, No. 10: 325–326. Oct., 1945.

The chemical composition and nutritional importance of the fatty acids of butter were determined. One volatile, two liquid, and two solid fractions were mixed with a basal ration of whole wheat flour, non-fat dry milk solids and salt and fed to rats. The effects on growth were compared with records obtained by feeding whole butter under similar conditions. To replace the butter Vitamin A destroyed in processing, raw carrots were included in the fatty acid diet. Supplemental vitamin D was given to all animals.

It was concluded that the fatty acid growth-promoting values were found in the liquid or unsaturated fractions, but that the volatile fatty acids were less efficient.

Relationship between body growth and the vitamin A content of the liver were observed. Those rats that were fed liquid fatty acids grew best and stored about twice as much vitamin A in their livers as the rats fed solid fatty acids. The rats that were fed volatile fatty acids grew least and stored least Vitamin A.

The preliminary reports indicate that the chemical composition of a food fat helps to determine the nutritive efficiency of other foods. P.S.L.

178. Greater Butter Values Shown. E. M. HARMON, Natl. Dairy Council, Chicago, Ill. Amer. Butter Rev., 7, No. 8: 238–239. August, 1945.

Nineteen states cooperating with the U. S. Bureau of Dairying and Office of Experiment Stations have partially completed a study showing that better feeding practices increase vitamin potency in butter.

Reports from fourteen cooperating states show that winter butter, which makes up 36% of all the creamery production in the United States, averages, per pound, 11,000 units of Vitamin A. Summer butter, making up 64% of all United States butter, averages nearly 18,000 vitamin A units per pound.

By carefully preserving feeds for winter dairy production so as to maintain the carotene, the vitamin A supplied by milk and milk products can be increased. While pasture grasses and roughage are principal sources of carotene, much of the carotene may be lost if care is not taken to preserve it.

It was also shown that storage and time lag in distribution does not materially reduce the content of vitamin A in butter. P.S.L.

CHEESE

179. Contributions to the Study of Rancidity in Canadian Cheddar Cheese. III. A Comparison of the Lipolytic Activity of Slight Rancid and First Grade Samples. O. R. Irvine, Ont. Agr. College, Guelph, Ont. Sci. Agr., *26,* No. 1: 7. Jan., 1946.

Lipase naturally present in raw milk has been suspected as a cause of rancidity in cheese. In order to learn whether slight rancid cheese would display greater lipase activity than normal, first grade cheese, samples of each type were comminuted into homogenized 37 per cent cream saturated with sucrose and incubated for 12 days at 37° C. Lipase activities as shown by increases in titratable acidity and acids volatile in steam were approximately equal in the two types of samples. It is concluded that the treatment given the fat globules prior to or during manufacture may have a greater influence on the rate of fat hydrolysis than lipase content. O.R.I.

180. Une Nouvelle Modification De La Méthode Au Citrate Pour La Determination De La Matière Étrangère Dans Le Fromage. (A New Modification of the Citrate Method for the Determination of Extraneous Matter in Cheese.) R. Thibodeau, Dairy School, St. Hyacinthe, Que. Le Quebec Laitier, *5,* No. 1: 8. Jan., 1946.

The citrate method for determining extraneous matter has been modified for use with a 15 g. sample rather than the larger samples previously recommended. A 15 g. sample of cheese is cut fine and then comminuted for one minute in a Waring Blendor with 200 ml. of 10 per cent sodium citrate solution at 150° F. It is then transferred to a conical flask and stirred for about 5 minutes and maintained at 150–160° F. The sample is then filtered through a filter pad held in a special suction funnel with a one-quarter-inch aperture. This filter pad is then dried, mounted and classified according to standards adopted from those of the State of Illinois. O.R.I.

181. A Study of the Balance of Certain Milk Nutrients in the Making of Cheddar, Cheshire and Stilton Cheese, and of Their Fate During the Ripening of the Cheeses. D. V. Dearden, K. M. Henry, J.

HOUSTON, S. K. KON AND S. Y. THOMPSON, National Institute for Research in Dairying, University of Reading, Reading, England. Jour. Dairy Res., *14,* 1 & 2: 100–116. 1945.

Cheshire, Cheddar and Stilton cheeses were made on one occasion from winter, and on another from summer, milk.

The distribution of vitamin A and carotenoids, vitamin B_1, riboflavin and vitamin C between milk, whey and curd was measured during cheese-making.

The fate of these vitamins during ripening was determined.

Some 80% of vitamin A and carotene, 15% of vitamin B_1 and 25% of riboflavin originally present in milk were recovered in the green cheese and persisted throughout ripening.

Vitamin C disappeared progressively during cheese-making and was absent from the cheeses.

Vitamin B_1 and especially riboflavin were recovered in cheese in larger proportions than would be expected from the partition of water between curd and whey, indicating that they were partially present in milk in combination with the coagulable solids.

The concentrations of the various vitamins in milk and the ripe cheeses were respectively for the winter experiment: vitamin A 15 i.u./g. fat and 13 i.u./g. fat; carotenoids 1.8 μg./g. fat and 2.0 μg./g. fat; vitamin B_1 33.5 μg./100 g. and 53 (Cheddar), 44 (Cheshire), and 80 (Stilton) μg/100 g; riboflavin 84 μg./100 g. and 300 (Cheddar), 260 (Cheshire), and 240 (Stilton) μg./100 g. For the summer experiment, the values were: vitamin A 28 i.u./g. fat and 25 i.u./g. fat; carotenoids 10 μg./g. fat and 9–10 μg./g. fat; vitamin B_1 42 μg./100 g. and 55 (Cheddar), 48 (Cheshire), and 75 (Stilton) μg./100 g.; riboflavin 128 μg./100 g. and 330 (Cheddar), 330 (Cheshire), and 360 (Stilton) μg./100 g.

There was no difference between the three types of cheese made in winter in biological value or true digestibility of the proteins for which values of 72 and 98 respectively were obtained at an 8% level of protein intake by the method of Mitchell.

Diets were blended from dried Cheshire, Cheddar, or Stilton cheese and from dried whey to approximate the composition of a sample of dried milk. These diets, when supplemented with iron, copper, and manganese and given as an exclusive diet, produced growth in rats as good as that obtained with the mineralized milk itself. S.T.C.

182. The Heat Resistance of the Bacteriophages of Cheese Starter with Observation on the Estimation of Phage Concentration.

AGNES A. NICHOLS AND J. Z. WOLF, National Institute for Research in Dairying, University of Reading, Reading, England. Jour. Dairy Res., *14,* 1 & 2: 93–100. 1945.

The heat resistance, in milk, of bacteriophages attacking strains of lactic streptococci isolated from cheese starters was determined. Active phages did not usually survive a temperature of 75° C. (167° F.) for 7½ minutes, but many phages were not destroyed by heating for 50–60 min. at 65–70° C. (149–152.6° F.) and most survived at 70° C. (158° F.) for 10–15 min.

S.T.C.

183. Observations of Cheese Starters with Reference to Bacteriophage and the Phage-Organisms Relationships of Strains Isolated.

AGNES A. NICHOLS AND J. Z. WOLF, National Institute for Research in Dairying, University of Reading, Reading, England. Jour. Dairy Res., *14*, 1 & 2: 81–93. 1945.

Frequent examination of the mother and bulk starters from several cheese factories during the 1942 season showed bacteriophage to be prevalent in some factories and apparently absent from others. Outbreaks of phage attack could not be correlated with heat treatment of the cheese milk, and were geographically widespread over the country.

The phage-organism relationships of these starter strains and their phage races were studied, and on this basis it was possible to arrive at the relationship and in some cases the identity of some of the strains circulating in commerce. It was found that for the isolation of some phaging strains, milk was a more sensitive medium than broth. Commercial starters appear to be composed of more than one distinct strain. S.T.C.

184. Bacteriophage Infection in Cheese Manufacture.

H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Research Institute (N. Z.), Palmerston North, New Zealand, Jour. Dairy Res., *14*, 1 & 2: 64–80. 1945.

Previous work in bacteriophage infection of starters as a cause of slow acid development in cheese manufacture is reviewed, and the present practices in vogue in New Zealand described. Air-borne phage seems to be the form of infection which is most insidious and most difficult to exclude. The spread of phages as dust or droplet infections seems to explain the gradual increase of trouble with a given starter culture within a single factory and also throughout an entire dairying district.

Besides the air-borne droplet infection, there may be daily reinfection of cheese milk from incompletely sterilized factory and farm equipment which comes into contact with the milk. Phage contamination from factory and farm equipment can be determined by the following method: Samples of 10 to 15 ml. of the milk in question are collected in sterile plugged tubes and pasteurized at 155° F. (68.3° C.) for 10 minutes. The samples are inoculated with three drops of an uninfected culture of the appropriate starter culture and incubated at 37° C. (98.6° F.). Slides are made at intervals, stained and examined under the microscope. In samples which contain phage, there is gradually increasing growth followed by lysis (indicated by partial or complete disintegration of the organisms).

Air-borne infection of the starter culture is guarded against by: (1) propagation of the starter in a building remote from the cheese factory; (2) maintenance of constant air pressure in the propagating room with filtered air; (3) filtration of air into the cultures through cotton-wool. Sealed vessels with inlets plugged with cotton-wool are used for mother cultures and cans with "water-seal" lids for the bulk starter. S.T.C.

185. Reactions and Properties of Annatto as a Cheese Colour, Part II. C. R. BARNICOT, Dairy Research Institute (N. A.), Palmerston North, New Zealand. Jour. Dairy Res., *14,* 1 & 2: 59–63. 1945.

A Lovibond tintometer was found to be suitable for estimating the coloring power of annatto extracts in dilute alkaline solution. S.T.C.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

186. Reflexions Sur La Législation Francaise Concernant Les Lait Concentrés Et Les Laits En Poudre. (Reflections on French Legislation Dealing with Concentrated and Powdered Milks.) A. TAPERNOUX, L'ecole vétérinaire de Lyon. Le Lait *23,* No. 221–223: 1. Jan.–Mar., 1943.

The fact that concentrated milk products are so highly regarded and widely used in infant nutrition places a special responsibility upon the manufacturer of these products. Standards of composition are reviewed and the proposal made that in the case of whole milk products, the milk used for manufacture contain at least 3.5 per cent fat and 12.35 per cent total solids. French regulations relating to these products are reviewed. O.R.I.

187. The Technique of the Bacteriological Examination of Dried Milks. CONSTANCE HIGGINBOTTOM, Hannah Dairy Research Institute, Kirkhill, Ayrshire, Scotland. Jour. Dairy Res., *14,* 1 & 2: 184–194. 1945.

It is recommended that the bacterial quality of spray-dried powders be determined after reconstitution at 50° C. (122° F.) by the plate count at both 37° C. (98.6° F.) (3 days) and at 30° C. (86° F.) (5 days). S.T.C.

188. The Effect of the Method of Reconstitution and of the Temperature of Incubation on the Plate Count of Spray-Dried Milk Powder. EDITH R. HISCOX, National Institute for Research in Dairying, University of Reading, Reading, England. Jour. Dairy Res., *14,* 1 & 2: 175–183. 1945.

Much higher counts were secured if the dry milk was reconstituted in a warm fluid rather than in a fluid at room temperature. Ringer solution warmed to 50° C. (122° F.) was found to give satisfactory results. Incubation of plates both at 37° C. (98.6° F.) and 30° C. (86° F.) was recommended. Many samples of powder contained microbacteria which do not grow at 37° C. (98.6° F.). They may so outnumber other organisms such as streptococci and micrococci, which grow at both temperatures, that the presence of the latter may be completely masked by incubation at 30° C. (86° F.). Even a short exposure of the poured plates to indirect sunlight was found to cause a decrease in the plate count. S.T.C.

189. Experiments on the Use of Antioxidants in Spray-Dried Whole-Milk Powder. J. D. Findlay and J. A. B. Smith, Hannah Dairy Research Institute, Kirkhill, Ayrshire, Scotland, and C. H. Lea, Low Temperature Research Station, Cambridge, England. Jour. Dairy Res., *14,* 1 & 2: 165–175. 1945.

The following substances were tested for antioxidant activity in laboratory-made spray dried milk powder: ascorbic acid, hydroxy-tetronic and dihydroxymaleic acids, gallic acid, and its esters, citric acid, potassium metabisulphite, gelatin hydrolysate, sodium pyrophosphate and creatinine, sodium hypophosphite, cysteine, tocopherol concentrates and a synthetic chroman and haematoxylin. Ascorbic acid and ethyl gallate proved most promising, ethyl gallate being the more powerful of the two. Ethyl gallate at a concentration of 0.07% increased the storage life of powder in accelerated tests 2½–3 fold. S.T.C.

190. Spray-Dried Milk Powder. Commercial Observations over Two Years of the Effect of High-Temperature Pre-Heating. E. L. Crossley, Alpin and Barrett, Limited, Yeovil, England. Jour. Dairy Res., *14,* 1 & 2: 160–164. 1945.

Pre-heating the milk to a temperature of 190° F. (88° C.) as compared with 165°F. (74° C.) was found to have only a very slight effect on the solubility of the resulting dry milk. The principal difficulty encountered in commercial operation was an increase in bacterial population after the first few hours of the run, due to multiplication in the evaporator and the atomizer feed tank. S.T.C.

191. Solubility of Non-Fat Dry Milk. S. T. Coulter, Univ. of Minn., Amer. Butter Rev., *8,* No. 3: 14. Feb., 1946.

The solubility of roller-dried powder is influenced by a number of factors including these three:

1. Completeness of removal of the dried milk film from the drum at each revolution. The rolls must be smooth and free from pits.

2. Temperature of the roll is dependent primarily upon the steam pressure used, but if this is superheated, reduction of pressure will not reduce temperature as much as might be expected.

3. The thickness of the film and the speed of the roller, even though adjustable, affect the solubility. Precondensing to not more than 18 to 20 per cent solids is a good practice.

A solubility index of 10 ml. is about as good as may be expected with atmospheric roller-dried non-fat milk solids. P.S.L.

DISEASE

192. Les Anémies D'Origine Lactée–Étiologie. (The Anemias Produced by Milk–Etiology.) A. HOUDINIERE. Le Lait. *23*, 221–223. Jan.–Mar., 1943.

Some aspects of this subject are reviewed. O.R.I.

FEEDS AND FEEDING

193. Effect of Storage on Carotene of Plant Extracts. A. R. KEMMERER AND G. S. FRAPS. Texas Agr. Expt. Sta., College Station, Texas. Jour. Ind. Eng. Chem., Ind. Ed., *38*, No. 4: 457–458. April, 1946.

Experiments were conducted to ascertain the relative changes during storage in the percentages of the isomers and other constituents of the crude carotene of plants. Thirteen samples of dried grasses and dried foods stored up to 8 months, showed an increase in the percentage of impurity A in the crude carotene fraction. The percentage of β-carotene in the pure carotene fraction decreased in 6 samples and remained nearly constant in the other 7. The percentage of neo-β-carotene U in the pure carotene increased in 11 samples and that of neo-β-carotene B decreased in 10. The quantity of impurity A increased in dehydrated carrots up to 14 months of storage and in beet greens up to 4 months of storage. In all other samples, there was a decrease in the amount of impurity A. β-carotene, neo-β-carotene U, and neo-β-carotene B decreased in all samples except beet greens, in which there was a slight increase in neo-β-carotene B during the second and fourth months of storage. B.H.W.

194. The Effect of the Diet on Foetal Development. L. R. WALLACE, School of Agriculture, Cambridge. Proc. of Physiol. Society, Jour. Physiol., *104*, 3: 34 p. Jan., 1946.

By feeding some ewes on a much-restricted ration during the last weeks of pregnancy, 144-day-old foetuses were obtained whose mean weight averaged only 57% of those obtained from liberally fed ewes. Under the conditions of this experiment, the growth of flesh (muscle and fat) is depressed more than the growth of nerve tissue and bone. Within the skeleton there was no, or at most only a slight, differential effect.

As with the major tissues, so with the organs. The alimentary tract was less affected than the heart, the heart less than the lungs and kidneys, and those in turn less than the thymus, spleen and liver. D.E.

FOOD VALUE OF DAIRY PRODUCTS

195. **Observations on the Calcium Content of Cow's Milk.** E. M. SEMMONS AND E. W. McHENRY, School of Hygiene, University of Toronto, Toronto, Canada. Canad. Jour. Public Health, *37,* No. 1: 22. Jan., 1946.

The calcium contents of milk samples from a large Toronto dairy, secured from time to time during two months, averaged around 100 mg. per 100 g. This value is 20 per cent less than the amount cited in standard tables of food composition. Pasteurization was shown not to be responsible for the low value found. It would appear that either milk available in the Toronto area has a subnormal calcium content or that the calcium content given for milk in most food tables is erroneous. O.R.I.

196. **Comparison of the Growth-Promoting Value for Rats of Butter Fat, of Margarine Fat, and of Vegetable Oils.** K. M. HENRY AND S. K. KON, National Institute for Research in Dairying, University of Reading, Reading, England and T. P. HILDITCH AND M. L. MEARA, Department of Industrial Chemistry, University of Liverpool. Jour. Dairy Res., *14,* 1 & 2: 45–48. 1945.

In a series of experiments with rats, the growth-promoting properties of butter fat were compared with those of the solid and liquid fractions of butter fat with margarine and with the following vegetable oils: maize, arachis, cottonseed and soybean. The separation of butter fat into fractions containing a preponderance of glycerides of saturated or unsaturated acids was effected by means of crystallization from acetone. The fats were incorporated into liquid skim milk for feeding.

The authors summarized their results as follows:

(a) No differences were detected between the growth-promoting properties of butter and margarine or of butter fat and deodorized arachis oil; in the latter comparison the arachis oil was more economically used than the butter fat.

(b) No differences were found between butter fat and maize oil alone or containing the solid or liquid fractions of butter fat in the approximate proportions in which they occur in the original fat. The less saturated oils, *i.e.*, maize oil and maize oil + the liquid butter fraction, were more economically used than butter fat and its solid fraction mixed with maize oil.

(c) No differences were observed between the growth-promoting values of butter fat, arachis oil, cottonseed oil or soya-bean oil. The butter fat was rather less well utilized than the less saturated oils, but the differences were not significant.

(d) Poorer growth was observed with the more saturated fraction of butter fat than with the original fat or the liquid fraction.

When butter fat, margarine fat or arachis oil was incorporated in a dry basal diet, no differences were observed in the growth-promoting value of these fats, but the arachis oil was more economically used than the other fats. This finding was not confirmed in a second experiment of longer duration in which poorer growth was observed with the arachis-oil diet, though the economy of gains was the same with all diets.

It is concluded from these experiments that it is unlikely that butter fat possesses superior nutritive properties to those of other fats, and that the more saturated fraction of butter fat is certainly not superior in growth-promoting value to that of the more unsaturated fraction or to more unsaturated vegetable oils. S.T.C.

ICE CREAM

197. Composition of Commercial Ice Cream. E. A. Grant and W. C. Henry, Ontario Research Foundation, Toronto, Canada. Canad. Jour. Public Health, *37*, No. 1: 27. Jan., 1946.

Analysis on 14 samples of ice cream from 5 Ontario cities revealed the following average values: Protein 5.0%; Fat 10.3%; Carbohydrate 17.6%; Calcium 150 mg./100 g; Vitamin A 520 I.U. per 100 g.; Thiamin 65 μg. per 100 g.; Riboflavin 240 μg. per 100 g. O.R.I.

198. The Story of How Vitamin-ed Ice Cream was Developed. Max A. Joffe, Head of Joffe Laboratories, Los Angeles, Calif. Ice Cream Field, *47*, No. 3: March, 1946.

It is stated that the formula whereby vitamins A, D, and E as well as the B complex vitamins—nine in all—are prepared and incorporated in stable form in ice cream was developed in the Joffe Laboratory, and that various aspects of the problem are covered by patent applications.

The author considers ice cream an ideal medium through which vitamins may be made available to the public. One quart of the vitamin-ed ice cream produced under the formula mentioned "furnishes the daily requirements of food supplements and does not act as a medicine" it is claimed.

W.C.C.

199. The Effect of Freezing and Cold Storage Upon the Bacterial Content of Egg Melange. C. K. Johns and H. L. Berard, Science

Service, Dom. Dept. of Agr., Ottawa, Canada. Sci. Agr., *26,* No. 1: 34. Jan., 1946.

Freezing egg melange brought about a sharp reduction in the numbers of bacteria present in whole egg melange. In two series of tests involving 44 samples, the average reduction in count was nearly two-thirds. Subsequent storage at 5° F. for 6 months resulted in a little further change in count, although *Escherichia coli* appeared to die off after three months.

In a comparison of methods of estimating bacterial numbers it was found that direct microscopic counts were always lower than the plate counts at 30° C. for some unexplainable reason.

O.R.I.

200. Frosted Foods More Than an Ice Cream Sideline. H. B. GRANT, Publisher of Ice Cream Field and Frosted Food Field. Ice Cream Field, *47,* No. 3: 12. March, 1946.

The author cautions against considering frosted foods a "sideline." He states that in contrast to the aim of the ice cream industry of a billion gallon annual production, which is approximately double present volume, the frozen food industry is aiming for 40 times present volume, *i.e.,* an eleven billion dollar annual business.

It is predicted that soon, complete pre-cooked meals will be frozen. By way of further contrast, it is pointed out that an ice cream retailer speaks of 6 to 10 flavors whereas the frozen food center is stocked with from 25 to 150 different frozen food items. An instance is cited where in one frozen food center, ice cream is sold, along with other frozen food items, at 50 cents per pint.

It is predicted that one super-market will sell more ice cream out of one frozen food cabinet than ordinarily is sold by ten retail shops in the same neighborhood. The ice cream manufacturer should seek to sell package ice cream through these super-markets and he is more apt to do this, the author claims, if he also services the cabinets with frosted foods. An ice cream manufacturer who expects to distribute frosted foods must be prepared to compete with other distributors of frozen foods, but it is concluded that combination of ice cream and frosted foods can be merchandized profitably.

W.C.C.

MILK

201. Le Dosage De La Materière Grasse Des Crèmes. (Determination of Fat in Creams.) J. PIEN, Directeur des Laboratoires de la Laitierie des Fermiers Reunis. Le Lait, *23,* No. 221–223: 18. Jan.–Mar., 1943.

Comparative results on two methods of determining the butter fat content of cream are given for 10 samples of sweet cream as well as on the same samples after holding for 10 days at room temperature. One industrial method involving a modification of the Gerber test appeared to give as satisfactory results as the Gottlieb centrifugation method. O.R.I.

202. **Square Bottles.** C. D. Kelley, Borden Company, Gridley Division, Milwaukee, Wis. Internatl. Assoc. Milk Dealers Bul., *38*, No. 4: 103–106. April, 1946.

The following summary of experiences in the change-over and the use of the square bottle is given:

1. Costs in our change over were minor.
2. No equipment changes necessary in our Milwaukee operation.
3. Breakage no greater than with round bottle.
4. Trippage. Slightly better.
5. Space saving is the outstanding advantage and on the whole the advantages far surpass the disadvantages. E.F.G.

203. **Sanitary Standards Subcommittee of the Dairy Industry Committee.** T. J. Kullman, Committee Member and Production Manager, Bowman Dairy Co., Chicago, Ill. Internatl. Assoc. Milk Dealers Bul., *38*, No. 4: 107–113. April, 1946.

A. Milk storage tank specification in process of development.

B. Sanitary fittings—details given—(not possible to summarize).

C. Shipping cans—details given.

D. Milk transportation—tentative minimum standards now being drawn.

E. Weigh cans and drop tanks—first draft of sanitary standards has been prepared.

F. Milk pumps, homogenizers, electric motors, milking machines, pails and strainers under consideration. E.F.G.

204. **The Effect of Temperature of Pre-Heating, of Clarification and of Bacteriological Quality of the Raw Milk on the Keeping Properties of Whole Milk Powder Dried by the Kestner Spray Process.** A. T. R. Mattick and Edith R. Hiscos, National Institute for Research in Dairying, University of Reading, E. L. Crossley, Aplin and Barrett, Ltd., Yeovil, C. H. Lea, Low Temperature Research Station, Cambridge, J. D. Findlay and J. A. B. Smith, Hannah Dairy Research Institute, Kirkhill, Ayr, and S. Y. Thompson and S. K. Kon, National Institute for Research in Dairying, University of Reading, with an appendix by J. W. Edgell, University of Bristol. Jour. Dairy Res., *14*, 1 & 2: 116–159. 1945.

Part I. Keeping Properties: Factors Influencing Stability. Increasing the pre-heating temperature of the milk from 165 to 190° F. (74 to 88° C.)

before spray-drying greatly improved the resistance of the powder to the development of tallowy "off" flavors on storage. Improvement in the bacteriological quality of the milk supply and more careful control during its passage through the plant produced some improvement in the keeping properties of powders prepared from milk pre-heated at the lower temperature, but had relatively little effect on the keeping properties of the powder when the higher pre-heating temperature was used.

The shapes of the deterioration curves for the various powders showed that the effect of the higher pre-heating temperature and of improvement in the milk supply was to extend the induction period which precedes rapid deterioration. Clarification did not significantly increase the storage life of the powder.

Part II. The Effect of the Various Factors Upon the Bacterial (Plate) Count of the Intermediate Products and of the Final Powder. The plate count of milk powder was significantly reduced by improvement in plant management and in cleaning and improvement in the milk supply. Plate counts of stored powder were higher in plates incubated at 30° C. (86° F.) than in those incubated at 37° C. (98.6° F.). Based on this work, the factors which determine the plate count of dried milk appear to be, in the order of their importance; (1) plant cleanliness and sterility, (2) pre-heating temperature, (3) bacteriological quality of the raw milk supply. The flora surviving in dry milk powder comprises streptococci, micrococci and spore-bearing organisms. When the milk was pre-heated to a temperature of 165° F. (74° C.), micrococci predominated over streptococci, whereas when the milk was pre-heated to 190° F. (88° C.), streptococci predominated and spore bearers were relatively numerous.

Part III. Chemical Enzyme and Vitamin Determinations. The effects of various processing treatments on vitamins A, B, and C and on carotenoids and riboflavin of the various spray-dried milks was studied in the course of a year's storage. Pre-heating the milk to 190° F. (88° C.) was not more deleterious to the vitamins studied than pre-heating to 165° F. (74° C.). The stability on storage of vitamin A and carotenoids and of vitamin C in powders pre-heated to the higher temperature was enhanced. Gas packing afforded further protection. After reconstitution there occurred on standing in the dark in all samples of milk, a certain progressive loss of vitamin C. This was much more marked in the samples pre-heated to 165° F. (74° C.). S.T.C.

205. The Resazurin and Methylene-Blue Tests as a Measure of the Keeping Quality of Milk. E. B. ANDERSON AND G. S. WILSON, Central Laboratory, United Dairies, Ltd., London, and Emergency Public Health Laboratory, Oxford, England. Jour. Dairy Res., *14,* 1 & 2: 21–27. 1945.

A comparison was made of (a) the 10-minute resazurin test, (b) the

1-hour resazurin test, (c) the resazurin test in which the time to reach disk 1 was recorded, (d) the modified methylene-blue test, and (e) a keeping quality test based on taste and alcohol precipitation. Altogether 2588 samples of raw milk were examined during four periods equally spaced throughout the year. The time required for the milk to acquire an abnormal flavor or a positive alcohol test was used as an estimate of the keeping quality. The correlation between the keeping quality test and the various indirect tests were as follows: 10-min. resazurin and K.Q., 0.45: 1-hr. resazurin and K.Q., 0.59: time to reach disk 1, resazurin and K.Q., 0.69 methylene-blue reduction and K.Q., 0.70. It was concluded, therefore, that the methylene-blue test gave the best indirect index of the keeping quality of raw milk, although the time to reach disk 1 in the resazurin test was very nearly as accurate. S.T.C.

206. Temperature Control in the H.T.S.T. Pasteurization Process. J. A. HALL, Physics Division, National Physical Laboratory, Teddington, England. Jour. Dairy Res., *14,* 1 & 2: 1–20. 1945.

An investigation was made into the accuracy and speed of response of temperature measuring instruments suitable for use in the control of H.T.S.T. pasteurization. Thermocouples used in conjunction with a "d.c. amplifier" recorder system was shown to be markedly superior to both vapor-pressure and mercury-in-steel thermometers. The accuracy of temperature measurement was about ± 0.3° F., as against ± 1° F., while the lag constant was about 0.2 seconds as against about 3 seconds. Response to small, rapid, and transient changes of temperature were shown to be greatly improved. The operation of control contract by the thermocouple recorder was found to be reliable to well within the accuracy of calibration, but the other type showed variations of the order ± 1° F. in addition to the uncertainty of calibration. S.T.C.

207. Daily Advertising Survey. T. P. KENDALL, Nat'l Research Bureau, Inc. Amer. Milk Rev., *7,* No. 9: 345–346. Sept., 1945.

As a result of the survey, a monthly appeal index was charted which permits advertising managers to plot the changes in appeal trends for milk. A "Wheel of Advertising Appeals Among Dairies" was transformed to a chart showing how dairies spent their advertising dollar. It was based upon 250 advertisements of milk covering a nine-month period between July, 1944, and April, 1945.

Percentages appear in following order: (1) Child appeal 15%; (2) Freshness, taste 8%; (3) Health 14%; (4) Institutional 23%; (5) Nutrition 10%; (6) Quality 23%; and (7) Miscellaneous 7%. P.S.L.

208. A Comparison of the Cleaning of Square and Round Milk Bottles Under Regular Commercial Conditions. T. V. Armstrong and L. H. Burgwald, Ohio State Univ., Columbus, Ohio. Amer. Milk Rev., *8*, No. 1: 34, 35, 36, 37. Jan., 1946.

This study was conducted in a city of 300,000 served by about 30 milk distributors. Washed bottles were obtained from seven different makes of washers in nine of the plants in this market, during the months of June, July, August, and September, 1945. The bottles used were collected from typical representative routes.

Two groups were recorded, with and without a chlorine rinse. The plate count method of determining bacterial content was used. Quart and half-pint bottles were examined and the counts were recorded per bottle rather than per ml.

Of the square bottles 4.3 per cent had counts in excess of 1,000 per bottle and 7.3 per cent of the round bottles had a similar count. Of the square bottles 1.1 per cent contained specks of dirt and 5.3 per cent of the round bottles had specks. Of the half pint bottles 9.6 per cent of the square type had counts of 250 or more per bottle while 8.6 per cent of the round bottles were so classified. No visible dirt was observed in any of the square half pints and 8.6 per cent of the round half pints had dirt specks.

The authors conclude that there is no difference in the commercial practicability of cleansing and sterilizing in typical dairy soaker equipment of the returnable square milk bottle and the conventional round bottle.

P.S.L.

209. Marketing Efficiencies as a Part of Our Post-War Economy. Ezra Merrill, Marketing Specialist, H. P. Hood and Sons, Boston, Mass. Amer. Milk Rev., *7*, No. 8: 310, 312. Aug., 1945.

Six economies in distribution practices effected during the war were: Every-other-day daylight retail delivery; six-day delivery of wholesale milk with no call-backs; discontinuation of all special delivery; elimination of bottling pints; split stops limited to not more than two dealers; and deposit charges for bottles, cases and cans. Every-other-day delivery has been the most important economy ever introduced in the distribution of milk. The increase in consumption has had an effect upon this economy since cost per quart goes down as volume goes up.

The adoption of every-other-day delivery reduced the total mileage of the truck by an average of 40 per cent, about 50 cents a day, or 0.0015 per quart. It brought about a great saving in delivery time, this being nearly halved per quart. At the same time, route loads increased nearly 40 per cent.

The author estimates that a return to pre-war practices would mean at least a one cent per quart increase in price. Increases in price will invite inroads by competitive products such as canned milk. The fluid milk dealers might well consider the continuation of these economies in their post-war plans. P.S.L.

210. Sonic-Soft-Curd Vitamin D Milk. ANONYMOUS. Amer. Milk Rev., 7, No. 8: 286–288. Aug., 1945.

The heart of the system is the sonic oscillator which may be described as consisting of two stainless steel circular plates between which the milk is pumped in a thin film at the bottom and out at the center. Behind one of the stainless steel plates is an oscillating electrical magnet of high voltage that sets up a vibration in the diaphragms, resulting in intense waves of energy at the rate of 360 per second. The result is a breaking up and dispersion of fat globules similar to that in homogenized milk. P.S.L.

211. Certain Defects and Faults in Market Milk By-Products. R. J. RAMSEY, Ramsey Labs., Cleveland, Ohio. Amer. Milk Rev., 7, No. 8: 278, 280, 282, 284, 313. Aug., 1945.

Defects of cream are listed as: (1) poor keeping quality, (2) lipase action, (3) cream plug, (4) cream line, and (5) air incorporation during bottling. The first consideration is high quality milk to start with, and adequate pasteurization. Particular precautions to prevent lipase action include use of hold over milk, preheating before separating, separating at 110° F., care that the cream is not agitated excessively and prompt pasteurization. To avoid cream plug, avoid use of frozen milk or partially churned milk. Avoid extremes in temperature. Cream should not be repasteurized nor should "returns" be used. Cream should be iced for delivery in warm weather.

Soapiness in homogenized milk can be avoided by careful pasteurization before homogenization. Sediment can be eliminated by clarifying. Cooked or chalky flavors occur when pasteurization is above 145° F. for 30 minutes.

Homogenizing cream eliminates cream plug, cream line, adds coloring powers in coffee, lends a richer flavor, and leaves a cleaner bottle after pouring. Twenty per cent cream should be homogenized at about 500# (300–750#) pressure and pasteurized at 150° to 165° F. 30 minutes. Homogenized cream does not whip well.

Cottage cheese is more perishable than fresh milk and only extreme care on the part of the manufacturer and distributor will insure increased consumption. Use only milk of good quality, pasteurizing at 145° F. 30 minutes: Use enough of a good active starter to ripen the cheese in 12 hours. Creaming should be done with care and delivery with dispatch. P.S.L.

212. Wartime Economies in Milk Delivery. DR. LELAND SPENCER, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., 7, No. 10: 370. Oct., 1945.

During the war the prices paid for milk by consumers increased much less than did the returns to producers. This was true despite significant advances in wages of persons employed in distribution and in prices of fuel, gasoline, tires, etc.

Two unusual developments which made this possible were: (1) Payment of substantial subsidies by the Federal government. (2) A remarkable lowering of milk distribution costs. The principal change was the adoption of every-other-day service to retail customers. In addition, second calls and special deliveries were eliminated.

The conclusion was drawn that, if pre-war type of delivery were reinstated, using 1945 wage and commision rates, cost of delivering milk to retail customers would increase 1.3 cents per quart, but on 1942 wage and commission rates the increase would be 6.7 cents per quart. On the basis of present wage and commission schedules, return to pre-war methods and loads would reduce earnings of route men $3.00 per week. It would necessitate increase to $\frac{1}{3}$ more routes.

Whether the cost-saving plan of delivery service now in effect or the pre-war type would yield greater returns to producers depends largely upon the attitude of route consumers. Little is known of this factor. P.S.L.

213. Milk Delivery in War and Peace. LELAND SPENCER AND H. ALAN LUKE, Cornell Univ., Amer. Milk Rev., 7, No. 12: 466, 468, 470, 472, 496, 498. Dec., 1945.

The data for this study were obtained from a survey by questionnaire sent to dealers distributing milk in the New York market. They covered the periods of May, 1942, and October, 1944.

Economies deemed most important effected by the federal government during the war were: (1) Delivery to retail customers was limited to every other day. (2) Elimination of Sunday deliveries to wholesale customers. (3) No special deliveries or call-backs. (4) Advance orders only by wholesale and retail customers. (5) Wholesale customers not allowed to return unsold products.

The entire survey is summarized by the following: Less than one-fourth of the milk distributed in New York City is delivered to consumers' homes. However, in suburban area, more than two-thirds of the milk is delivered to retail customers.

Three-fourths of the milk dealers in New York market sell less than 1,000 quarts a day. Five per cent of the dealers are of this small group. Five dealers sell 100,000 quarts per day and, as a group, handle 55 per cent of the total volume of the market. The largest distributors have the largest loads and shortest routes. Wholesale routes do not vary so much in volume.

Between May, 1942, and October, 1944, the average quantity of milk delivered per route per day increased 50 per cent, and mileage was reduced 35 per cent. For wholesale deliveries there was a 7 per cent increase in size of loads, but delivery vehicle mileage was reduced 22 per cent. Quantity of milk delivered per mile was increased 54 per cent on retail routes and 42 per cent on wholesale routes. Dealers, who responded to the questionnaire sent them, favored retention of every other day delivery and favored state or federal regulation to effect it.

The authors state that a change of conditions from war to peace could change reactions surveyed at a time when the country was at the peak of its war effort. The success of the war-time practices depended upon the cooperation of consumers and delivery men as well as distributors. P.S.L.

214. Pasteurization by High Temperature—Short Time. W. W. CABANAUGH, York Corp. Amer. Milk Rev., 7, No. 9: 324, 353, 354. Sept., 1945. Amer. Milk Rev., 7, No. 10: 378, 380, 382, 384. Oct., 1945.

High-temperature-short-time pasteurization was introduced in the late 1920's. However, it was not until the adoption of the heat exchange plate that the method became widely acceptable. Hot water instead of electricity is used for the final heating of the milk. This, coupled with the fact that pasteurization is done close to the temperature at which cream line becomes affected, made it necessary to develop control apparatus that would positively regulate milk temperature between very narrow limits. This apparatus must also control a milk pump stop in the event below-temperature milk leaves the heater.

Conclusions of the report made on a study of performance of the method showed: 1. Results obtained indicate that heat is the destructive agent and that hot water and electricity are equally efficient in pasteurization of milk. 2. The results indicate an ample margin of safety in time and temperature when the milk is heated to 160° F. and maintained at that temperature for at least fifteen seconds. 3. Operation of milk pump cut-out is necessary as a protection against the treatment of milk at temperatures below 160° F. 4. Hemolytic streptococci evidently survive flash heating better than tubercle bacilli. 5. The York Plate Pasteurizer meets the structural, equipment and operational recommendation set forth in the 1931 report of Committee on Milk Supply Eng. of A.P.H.A. The York equipment includes a thermal limit recorder controller which records the temperature of the milk, leaving the pipeline holder; records the frequency and duration of operation of the flow diversion valve, and operates the flow diversion valve through actuation of a solenoid valve releasing air pressure when it is in diversion flow.

The author reports that there is virtually no limit to the capacity of units, some of them operating for 22 hours daily. The time required for milk to flow from storage tank through the pasteurizer to filler is 1½ minutes.

It is recommended that sufficient storage facilities be provided for at least one hour of continuous operation and that, for long runs when filtering hot, two filters be used. Clarifying or filtering may be done hot or cold.

With high-temperature-short-time pasteurization, thermophylic bacteria do not cause trouble even on prolonged runs. Thermoduric bacteria, however, are not appreciably reduced. Milk retains its raw milk flavor and cream line is comparable to lower temperature holding system.

Advantages of this method include: less labor required for operation and cleaning; time reduction between dumping room and fillers; small amount of floor space required; closed cycle avoids evaporation and re-infection; apparatus is flexible; operation is practically automatic; pasteurization is uniform; cost of pasteurization is lower. P.S.L.

215. Consideration on the Keeping Quality of Pasteurized Milk. L. H. BURGWALD AND D. V. JOSEPHSON, Ohio State Univ., Columbus, Ohio. Amer. Milk Rev., *8*, No. 1: 46–47. Jan., 1946.

A total of 184 bottles of milk, from eight different lots, were tested for keeping quality in these trials. The usual grades of pasteurized milk will stay sweet for seven to twenty-eight days under the normal conditions found in homes equipped with refrigerators. The milk was considered sour when it developed 0.03 per cent or more acidity. Treatment of the milk and temperature of the refrigerator influenced spoilage more than bacterial counts at the beginning of time tests.

Half-pint samples, under similar conditions, stayed sweet an average of 21.8 days. Three sets of quart bottles of milk were taken from each lot, and the first was placed in a refrigerator immediately, the second was exposed at the same temperature for two hours. The average temperature of No. 1 was 43° F. and the set remained sweet for 12.3 days. Set No. 2 had an average temperature of 61.5° F. when the samples were placed in refrigeration and kept at a temperature of 43° F. Set No. 3 had an average temperature of 43.1° F. and remained sweet for an average of 12.4 days. The quart samples were taken out of the refrigerator for five to seven minutes while tests were run. This resulted in an increase in temperature of 2–4° F.

Initial bacteria counts did not indicate milk's potential keeping qualities. One lot of samples had an initial count of 61,000 per ml. and remained sweet for 25 days. Another had 7,150 bacteria per ml. and stayed sweet for only 16 days. There was no appreciable increase in bacteria count up to four days; however, after four days, psychrophilic bacteria increased rapidly. There were no changes noted in flavor until the samples were from four to seven days old. Even then, these were only slight and were not objectionable.

Vitamin C varied considerably in the samples and deteriorated very rapidly. Riboflavin was comparable in all samples and remained practically constant throughout the tests.

Refrigeration temperatures for milk storage should be below 45° F. and preferably at 40° F. P.S.L.

216. **Keeping Quality of Milk.** L. H. Burgwald and D. V. Josephson, Dept. of Dairy Technology, Ohio State Univ., Columbus, Ohio. The Dairy World, *24*, No. 11: 36. April, 1946.

See Preceding Abstract. F.J.D.

217. **Keeping Quality of Pasteurized Milk in N. Y. Area.** A. C. Dahlberg, Dept. Dairy Indus., Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *7*, No. 10: 372–374. Oct., 1945.

See: Jour. Dairy Sci., *28*, No. 10: 779–792. Oct., 1945. P.S.L.

PHYSIOLOGY

218. **Reviews of the Progress of Dairy Science.** Section A. Physiology of Dairy Cattle. I. Reproduction and Lactation. J. A. B. Smith, Hannah Dairy Research Institute, Kirkhill, Aryshire, Scotland. Jour. Dairy Res., *14*, 1 & 2: 195–224. 1945.

This is a review of recent literature in the field. 358 references. S.T.C.

MISCELLANEOUS

219. **Mise En Evidence Et Isolement Des Enduits Protecteurs De L'Aluminum. (Detecting Aluminum Foil Coatings.)** M. H. Couturier. Le Lait, *23*, 221–223. Jan.–Mar., 1943.

Coated, laminated aluminum has replaced tin foil as a packaging material for many food products. The resistance of aluminum to certain chemical agents is limited, however, and its use is dependent upon synthetic coatings. Imperfect coating of the metal can be detected by several tests which include: 1. Plunging in aqua regia, 2. Treatment in a 1% solution of bichloride of mercury and a 10% solution of phosphoric acid, 3. Removal with organic solvents. A more rapid test consists of using a 5% solution of bichloride of mercury, unprotected foil being "devoured" in less than one minute. O.R.I.

220. **Plans for Dairy Equipment—Post War.** A. W. Farrall, Michigan State College, East Lansing, Mich. Internatl. Assoc. Milk Dealers Bul., *38*, No. 4: 95–102. April, 1946.

Mechanization of many operations will result from difficulty in obtaining skilled hand labor, to remove human drudgery from plant operation and to improve operating efficiency, as for example with more automatic operating controls.

There is a larger use of conveyors, etc. Stainless steel can now be worked and machined more readily. Chrome plating and heat treatment has helped to lengthen the life of wearing parts. Dairy machines are constructed for closer tolerances resulting in finer machinery with better performance.

Automatic control will be more generally adopted for steam boilers, pasteurizers, refrigeration rooms, etc., resulting in reduction of costs and labor and a more satisfactory product. There will be a more general use of continuous rather than batch processes. Progress will be made in designing more machines to be completely dismantleable for cleaning. More progress will also be made in standardization of sanitary pipes and fittings and other small items.

On the farm the use of the separate milk house will increase and will be an aid to better quality product and easier care of equipment. In warmer climates especially the use of the refrigerated truck will likely increase rapidly.

For the plants there will be greater use of small fully automatic boilers, new can washers for use of new cleaners, water softeners for can washers, improved stainless steel pumps, bottle washers for new type cleaners, the new square bottle and plate heat exchangers. Quick frozen milk and ice cream offer possibilities. The continuous churn offers real possibilities for reduction of cost of processing combined with improvement of quality and uniformity of product.

In refrigeration greater use will be made of low pressure refrigerants. In plants where cost of land is not excessive the single working floor type is increasing with greater emphasis upon the use of new building materials and sanitation.

With the great amount of equipment now in dairy plants, a competent engineer and the training of employees to service equipment will assume greater importance. E.F.G.

221. **The Efficient Use of Fuel.** Prepared under the Direction of the Education Sub-Committee of th Fuel Efficiency Committee of the Ministry of Fuel and Power. Reprinted by permission of the Controller of his Britannic Majesty's Stationery Office. Chemical Publishing Co., Inc., Brooklyn, New York. 1945.

While the text is largely devoted to British practices, the essential fundamentals apply equally well in American practice and their very lucid explanation and development can be readily grasped. It is very complete in coverage of all phases of fuel use. Subjects of pertinent interest are composition and properties of all types of fuel, theories and principles of combustion, equipment used in burning all types of fuel, boiler efficiency and testing, water purification, auxiliary boiler equipment and boiler settings. Steam utilization for industrial processes, central heating, drying and evaporation are given detailed treatment. Also automatic control, the use of various necessary instruments, sampling coal and gases, and their analysis come in for a generous share of consideration. The thirty-four chapters of the book comprises 787 pages of textual matter which is elaborated very amply with 135 tables and over 303 figures, the latter chiefly drawings and a smaller number of graphs. It is important that fuel selection and utilization be given careful consideration in the dairy industry, a considerable user of steam, because of cost of fuel and its efficient use can be a very vital factor in the unit cost of processed milk or a manufactured milk product. It is the feeling of this reviewer that a book of this type could supply the information upon steam generation noticeably lacking in our excellent dairy technological books for much of its material is quite practical so that it may be grasped readily by the non-engineer. L.M.D.

222. How to Improve Coal Utilization and Boiler Efficiency. T. O. Phillips, Bituminous Coal Institute. Amer. Milk Rev., *8*, No. 1: 20–22. Jan., 1946.

It is the problem of the plant manager to select equipment that will burn coal with the best results. Stokers of either the underfeed chaingrate, pulverized coal burners, or spreader types may be used. The gasification of solid fuel and the complete combustion of the gas combine to achieve highest technical efficiency. Proper carbonation of the coal (mixing with air) is an important step.

Prevention of leaks include care that holes in the fire do not occur, that the doors not be opened to check the draft, and that too thin a fuel load not be carried. Incomplete burning of the coal and too much surface moisture should be avoided. Proper coordination of coal-burning equipment with the coal or coals which it will burn is very important.

In the purchase of coal, specifications may be obtained to determine B.T.U. content, quantity ash; and non-combustible materials. P.S.L.

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CONTENTS

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Diseases
Feeds and feeding
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Herd management
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Lancet
Le Lait

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Milk Industry
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ABSTRACTS OF LITERATURE

BOOK REVIEWS

223. **Secretion of Milk, Third Edition.** Dwight Espe. The Collegiate Press Inc., Ames, Iowa. 314 pp., illustrated.

Many of the readers of the Journal of Dairy Science will welcome the third edition of this important work. Those who have used the previous editions in their teaching or research work or who have it in their library as a source of information will appreciate the author's effort in keeping this book abreast of progress in a fast-moving field. The number of literature citations has been expanded to include 1,294 references which is almost double the number of the original edition which appeared in 1938. This is striking evidence of the interest and progress in the field. T.S.S.

224. **Water Bacteriology.** S. C. Prescott, C.-E. A. Winslow and M. H. McCrady. 368 pp. 6th Ed., 1946. $4.50.

The sixth edition of this standard text and reference book on sanitary water bacteriology is organized almost like the fifth edition but is brought up to date and considerably enlarged, with three more chapters, 145 more pages and 46 more tables. To the authors of previous editions, Doctors Prescott and Winslow, has been added another eminent water expert, Dr. M. H. McCrady, who had much to do with the latest revision of the book. Again the discussion is built about the latest edition of "Standard Methods of Water Analysis." Both official and unofficial methods are analyzed and interpreted and special attention is paid to the differentiation of the various coliform bacteria and the use of selective culture media. Emphasis is placed on the quantitative expression of results of the presumptive test and an appendix includes tables for obtaining the most probable number. The latest methods for examination of sewage and sewage effluents and of shellfish are brought up to date.

This edition, like earlier ones, deals only with the public health aspects of water bacteriology and does not discuss the general bacteriology of natural waters.

All workers on sanitary water bacteriology will welcome this new edition as will teachers of the subject. W.C.F.

BACTERIOLOGY

225. **Thermoduric and Thermophilic Bacteria in Dairy Products.** W. E. Noyes, Canadian Dairy and Ice Cream Jour., *25*: 2. Feb., 1946.

Thermoduric bacteria are heat-resisting microorganisms, approximately 90% of which survive ordinary pasteurization temperatures. Thermophilic bacteria are not killed at pasteurization temperatures but actually grow at these temperatures. Thorough removing of milkstone and subsequent cleaning and disinfecting of all utensils is necessary for complete control of these bacteria. There are two classifications for milkstone in the plant: (1) presence of milkstone is due to faulty cleaning methods and (2) from the formations of milkstone due to daily operations of specific types of equipment. To eliminate milkstone the use of proper cleaning products is necessary and the personnel should be shown how to properly clean the equipment and keep it milkstone-free. Manufacturers' directions should be followed on cleaning plate type short-time pasteurizers. Can washers should be in good working order to eliminate this operation as a factor in milkstone formation. Milk should not be repasteurized, as heat-resisting and heat-loving bacteria thrive on this treatment. Other precautions are to: (1) eliminate any dead ends on the sanitary piping and (2) control foam on pasteurization equipment.

Chlorine products, both in the plant and on the farm are effective in eliminating high counts due to these organisms. If these bacteria are not found in the plant, look to the farm for potential breeding places in milking machine. H.P.

BREEDING

226. Studies on the Metabolism of Semen. 1. General Aspects. Occurrence and Distribution of Cytochrome, Certain Enzymes and Coenzymes. T. MANN, Molteno Institute, University of Cambridge (England). The Biochem. Jour., *39,* No. 5: 451–458. 1945.

The ejaculated semen of the ram, bull, and boar were used for the study but most of the results are reported for ram semen. Emphasis is placed upon the difference between the metabolic characteristics of the spermatozoa and the seminal plasma and a procedure for the separation of the two is included. It is demonstrated that the spermatozoa contain a complete cytochrome system. Some evidence is given for the presence of adenosinetriphosphate in the spermatozoa and that its function as a coenzyme is in some way connected with motility. Mention is made of deaminases and phosphatases. A.O.C.

227. Studies on the Metabolism of Semen. 2. Glycolysis in Spermatozoa. T. MANN, Molteno Institute, University of Cambridge (England). The Biochem. Jour. *39,* No. 5: 458–465. 1945.

The enzyme systems involved in the carbohydrate metabolism of spermatozoa are studied. It seems that the reactions involved resemble those which take place in other tissues and in yeast metabolism. A.O.C.

228. Die Haltung der Zuchtstiere. W. Engeler, Herdbook Office for Brown Swiss Cattle, Zug, Switzerland. 107 pp., 42 halftones, 20 graphs. Verbandscruckerei AG, Bern. 1943.

An account of the conditions and laws under which Brown Swiss bulls are kept in Switzerland. Besides decribing the ownership, feeding, management, fees for service, etc., there is an analysis of the vital statistics for bulls as revealed by the records in the herd-book office. These statistics are analyzed for such things as the age composition of the bull population, age when service begins, age when sold for slaughter, causes of slaughter, number of services per unit time, conception rates, number of offspring marked per year, etc. Breeding associations own 33% of the bulls outright and rent about 25%. The other slightly more than 40% are entirely in private possession. The associations buy 65% of their bulls at the national show, 34% at local shows or from local breeders, and 1% from other associations. The average age of the living bulls is 1.8 years. The average age of bulls when sold for slaughter is 3.4 years. The average bull makes 280 services during his lifetime, which is 12.3 per month of effective breeding use. On the average 31 calves per bull are marked by the herdbook officers; only 15% have more than 50 such calves each. The number of services is 1.44 times the number of different cows served but, since some of these cows do not actually calve, the actual conception rate would be lower than the 69% which this appears to equal. J.L.L.

229. Das Photographieren Landwirtschaftlicher Haustiere. Edited by W. Engeler. Schriften Nr. 5 der Schweiz. Vereinigung für Tierzucht. 24 pp. 17 illustrations. Verlag Benteli AG., Bern. Fr. 2.40. 1944.

A symposium of seven lectures given at a short course in animal photography held by the Swiss Society for Animal Breeding at the Strickhof school near Zurich in April, 1944. The brief articles cover such topics as the purposes of animal photography, suitable equipment, suggestions on the making of negatives and positives, proper placing of the animals and choice of backgrounds, and special details concerning photography of horses, cattle, and smaller animals. J.L.L.

230. Untersuchungen über die Inzucht- und Verwandtschaftsverhältnisse und ihre züchterischen Auswirkungen in Hochzuchtgenossenschaften des schweizerischen Braunviehs. W. Engeler and P. Indermühle. Herdbook Office for Brown Swiss Cattle, Zug, Switzerland. Landwirtschaftlichen Jahrbuch der Schweiz. pp. 954–980. 1944.

The Brown Swiss cattle born in 1940 or earlier in the breeding associations of Sachseln and Elm were studied to learn how much inbreeding had been done, what direction it had taken, and what the results had been. The average inbreeding was only 2.22% in Sachseln and 0.86% in Elm. The average relationship of the present male animals to each other was 6.6% in Sachseln and 5.4% in Elm. Since this is more than twice the inbreeding coefficient, it appears that the average breeder made some effort to avoid inbreeding.

Detailed study of Sachseln showed that about three-fourths of the inbreeding there was to two bulls, Baron and Figi, unrelated to each other and both used extensively several generations ago. Apparently the inbreeding was not deliberately intended as such, but resulted incidentally from a widespread striving to save and use the descendants of these two bulls. The present Sachselen animals carry 13.1% of the blood of Baron and 11.0% of the blood of Figi. From this it may be presumed that about one-fourth of the genes in the present Sachseln population come from these two individuals. No clearer relation was found between the conformation score and the intensity of inbreeding of the individual animals. Since the range in inbreeding was not wide, clear evidence of such a relation was hardly to have been expected unless the relation was very close. J.L.L.

BUTTER

231. Neutralization and Acidity Control in Creamery Butter. D. L. GIBSON, Canadian Dairy and Ice Cream Jour., *25*: 4. April, 1946.

The article gives: (1) a review of the proper procedure and sequence to follow in neutralization, and (2) a discussion of the underlying factors which control pH and acidity. It has been found that, all things being equal, butter with a pH ranging from 6.7–7.2 has the best keeping quality. To obtain proper pH, (1) the type of neutralizer used is one of the main points to be considered when neutralizing cream; and (2) the titration on a vat of raw cream should be done with extreme care and a standardized method followed. Other minor factors that may affect the pH are, (1) the season of the year may affect the titratable acidity; (2) the percentage of fat in the cream, and (3) the amount of dissolved carbon dioxide in the raw cream. The method for measuring the pH of butter serum is outlined. H.P.

232. New Buttermaking Processes Used in German Creameries. W. H. COOK, Canadian Dairy and Ice Cream Jour., *25*: 3. March, 1946.

The Fritz process continuous buttermaker is described and diagrammed. It consists essentially of three parts: (1) horizontal water-jacketed drum with paddles that revolve at 2,850 r.p.m.; (2) pressing chamber consisting of two worm screws rotating at 60 r.p.m., and (3) succession of single turn worm screws separated by perforated plates. For this machine the milk is separated at a temperature of about 110° F. to produce 45–50% cream. The cream is flash pasteurized and cooled to 40° F. in a holding tank. From this tank it is pumped to the constant level tank supplying the butter machine. The fat content of the butter is influenced by: (1) initial fat content of the cream; (2) temperature of the cream; (3) rate of flow; and (4) the adjustment of the orifice in the machine. The butter is not washed by this method. Small units have a capacity of 990 pounds per hour while larger units make almost two tons per hour.

Another process for continuous buttermaking is the Alpha process. It is essentially a two-stage centrifugal process. The incoming milk is brought to a temperature of 85° C. (185° F.) in a pasteurizer and is passed at this temperature to the first separator which delivers cream containing 30% butterfat. This fat-rich fraction is cooled continuously in an enclosed shell-and-tube cooler to a temperature of 65° C. (149° F.) and at this temperature is passed through another centrifugal separator that delivers a cream fraction containing 80% butterfat. This fraction is immediately cooled in a special cooler, fitted with a screw expeller to room temperature or lower, and is delivered as butter. The butter is not salted. The largest unit of this type produced 550 pounds per hour, and some dairies have two or three units. The butter differs somewhat in physical properties from butter produced in the churn. A picture of an automatic cheese-making machine which completes the cheesemaking process without manual handling of the product during processing is shown. H.P.

233. **Report of Studies on the "Winter" Flavor of Butter.** A. H. White and C. A. Gibson, Canadian Dairy and Ice Cream Jour., *25:* 3 and 4. Feb. and March, 1946.

Studies were made of the effect of the stage of lactation and methods of handling cream and milk on the lipase actions in cream in relation to the development of bitter or "winter" flavor in the cream and butter. When cows were fed a well-balanced ration containing succulent feeds and the milk and cream were handled according to the best recommended methods, stage of lactation was not an important factor in lipase activity and the development of "off" flavors.

Lipase activity in winter cream as indicated by titratable acidities of the cream, fat acid values of fresh butter, and the flavor quality of both cream and butter, was promoted and accentuated by cooling and rewarming the milk before separation and by the addition of warm cream to cold cream.

Considerable bacterial growth takes place in cream held at 35° F. or lower, especially when warm cream is added to previously collected cold cream, and air cooled at a temperature of approximately 30° F.

To obtain and maintain the highest flavor quality in winter cream and butter, milk should be separated as soon after milking as possible and each lot of fresh cream should be cooled before being added to cream previously collected and cooled. H.P.

234. La Graduation des Butyromètres a Crème Un Nouveau Modèle de Butyrometre. (Graduation of Cream Butyrometers. A New Model of Butyrometer.) JEAN PIEN. Le Lait, *24:* 234–236. 1944.

In the Gerber method of determining butterfat, samples are digested with sulphuric acid and amyl alcohol. For cream the sample may be weighed and diluted 10 times in order that a milk butyrometer may be used. This practice multiplies errors. Errors also result from calibration of glassware at 20° C. while it is actually read at much higher temperature, inaccuracies in reading the meniscus and destruction or removal of soluble fatty acids in the acid-alcohol-water phase. A new butyrometer designed to give results directly in fat per kilogram of cream using a 5-gram sample or alternatively, the richness of cream per liter of a 5-cc. sample, is described. O.R.I.

235. Donnees Techniques sur la Beurrerie Francaise. (Known Techniques Applied to the French Creamery.) A. HOUDINIERE. Le Lait, *24*: 231–233. 1944.

The methods used in assessing quality in French butter are outlined and the results of chemical and microbiological tests on 228 samples are presented. These indicate a need for greatly improved composition and pH control as well as more efficient pasteurization and better buttermaking methods. The reasons for defects are discussed and the necessity for improved training of buttermakers stressed. A more adequate inspection system is also advocated. O.R.I.

236. Etude de la dilution des crèmes et son influence sur la composition des babeures. (Study of Dilution of Creams and Its Influence on the Composition of Buttermilks.) JEAN PIEN, Le Lait, *23*: 224–226. 1943.

Watering cream is undesirable for several reasons but particularly where the buttermilk is to be dried. The author develops mathematically the effect of varying rates of dilution upon the composition of creams and upon the resulting buttermilks. O.R.I.

237. What's Ahead for the Butter Industry? H. A. BENDIXEN, State College of Washington, Pullman. Nat. Butter & Cheese Jour., *37,* No. 4: 42. April, 1946.

The potential butter market is large. Future competition will be keen.

Efficient production on farms and in factories can improve quality and effect economies in utilization of labor. Better machinery for churning, product handling, can washing and refrigeration will help. Plant costs may be reduced by production of such diverse products as dry skim milk and buttermilk, soluble casein and butter oil. Management will do well to improve its labor relations through vacations, insurance plans, recreational programs, safety devices, and rest rooms. W.V.P.

CHEESE

238. Why Canadians Are Low Cheese Consumers. P. H. Casselman, Canadian Dairy and Ice Cream Jour., *25:* 3. March, 1946.

The temperature zone climate in Canada is such that cheese does not lend itself to hot meals. However, if proper organization and education were used, cheese consumption could be greatly increased. In Canada in 1934, the per capita cheese consumption was 3.6 pounds while most other countries averaged (1932–1934) from 4.0 to 19.2 pounds per capita. H.P.

239. Utilization of Whey for Profit to Producer and Manufacturer. D. H. McCallum, Canadian Dairy and Ice Cream Jour., *25*: 3. March, 1946.

Whey must not be drained into rivers or pools to produce pollution of streams and create a public nuisance and health hazard. Whey can be made a source of revenue shared by both cheesemaker and patron. Whey solids consist of valuable proteins, milk sugar, minerals, and vitamins. Whey makes an excellent feed for hogs. It is worth about one-half as much as skim milk fed to hogs either on pasture or in dry lot. One hundred pounds of whey is equivalent to approximately one-quarter bushel of corn or in protein value to eleven pounds of barley. Clean fresh whey may be fed to calves. The whey should be pasteurized, obtained fresh each day and fed in clean pails (pasteurization is necessary if all milk does not come from tuberculin-tested cows). Whey can be used as a substitute for mineral acid or molasses in making grass silage. Three to five hundred pounds of whey should be used per each ton of grass. Dried whey can also be used as a human food in candy, processed cheese, cheese spreads to replace skim milk powder and in the manufacture of penicillin. H.P.

240. Utilisation des Sérums de Fromagerie et des Lacto-Pretéines dans L'alimentation. (Utilization of Cheese Factory Whey and Milk-proteins as Foods.) Jean Pien. Le Lait, *23:* 227–228. 1943.

The value of whey and its products as an article of diet is extensively reviewed, particular emphasis being given to the effect of acidity and neutralization of the whey upon mineral values. Experimental values with respect to the effect of heating wheys of varying acidities result a reduction in ash content. It is concluded that the use of whey products as human food should be increased, preferably in the form of whey cheese. The need for improved sanitary methods of handling and manufacturing whey products is stressed. O.R.I.

241. **Le Sérac (a whey cheese).** A. Houdinière. Le Lait, *23*: 229–230. 1943.

A description is given of the principles involved in the manufacture of a cheese similar to Ricotta. Sweet or neutralized whey from Gruyere or Camembert cheese is heated rapidly to within a few degrees of boiling, after which vinegar or sour whey is added and the curd which comes to the surface is collected and drained. It is usually sold fresh and may be mixed with condiments. The moisture content is 75–80%. O.R.I.

CHEMISTRY

242. **Differential Adsorption of Pepsin and Rennin on Foam.** G. Andrews and F. Schutz. Abstract of paper read at the proceedings of the Biochemical Society (London), Sept. 28, 1945. The Biochem. Jour., *39,* No. 5: li and lii. 1945.

"The relative adsorption on foam of these enzymes has been studied, not so much to find a new way of purification, but to show that by investigations of this kind differences in the surface activities of enzymes, present in the same solution, can be detected." Foaming was carried out by introducing nitrogen at a low rate through a gas jet with an opening of 50 microns diameter. Beginning with a solution with a 1:1 concentration of pepsin and rennin, a concentration of 7.4:2.6 was achieved. From this it is concluded that pepsin is considerably more surface active than rennin.

A.O.C.

243. **Rennet Hysteresis and the Calcium Phosphate of Milk.** G. T. Pyne, Dept. of Dairy Chemistry, University College, Cork (England). The Biochem. Jour., *39,* No. 5: 385–390. 1945.

When milk is heated the time required for coagulation by rennet is increased. This phenomenon is referred to as rennet hysteresis by the author. The cause is believed to be "a heat-induced displacement of the equilibrium between calcium phosphate in solution and the colloidal calcium caseinate–calcium phosphate complex," with the heat converting some of the soluble calcium and soluble phosphate to a less soluble colloidal complex. The reverse of this reaction on cooling (less soluble complex to the more soluble calcium and phosphate) is slow and only partly completed.

Conductivity measurements, made after the removal of most of the chlorides by dialysis, showed an interesting parallel trend to the time required for coagulation by rennet. A.O.C.

CONCENTRATED AND DRIED MILK: BY-PRODUCTS

244. **La Caseine du Soya (glycinine) est-elle de la Caseine? (Is "Casein" of Soya Bean Casein?)** MAURICE BEAU. Le Lait, *24*: 234–236. 1944.

The question is raised whether soya "milk" and soya "cheese" may legally be designated as such since milk, cheese, butter, cream, etc., are, in France, exclusively animal products from a legal point of view. The composition of soya protein and casein, while of similar elementary composition, differ considerably in their content of valine, proline, tyrosine, histidine and lysine. Methods of extracting the protein of soya beans vary considerably, however. Because of a lack of similar properties it is concluded that the term "casein" should not be applied to soya or vegetable proteins. The term "legumine" is suggested as a suitable one to designate the general class of proteins obtained from peas, beans, soyas, etc. O.R.I.

245. **Le Sechage de la Caseine par Rayonnement Infrarouge. (The Drying of Casein by Infra-red Irradiation.)** MAURICE DERIBERE. Le Lait, *23*: 229–230. 1943.

Experimental results are presented indicating that infra-red lamps are effective as a method for applying heat in the drying of casein. Curves are presented showing that casein curds lose up to 75% of their moisture in 3 hours when spread 2 cm. thick, and mashed every quarter hour. Irradiation was at the rate of 0.42 watts per sq. cm. A good product is produced and the method lends itself to continuous operations. On a cost basis it is competitive with tunnel drying. O.R.I.

DISEASE

246. **Le Rôle du Lait dans la Transmission de Quelques Protozooses. (The Rôle of Milk in the Tranmission of Some Protozoa.)** A. HENERY AND J. GUILHON. Le Lait, *24*: 231–233. 1944.

The literature relating to the transmission of such protozoa infections as Trypanosomosis, Leishmaniosis, Spirochetosis, etc., is reviewed. Milk other than that of the bovine species is involved in many instances. O.R.I.

247. **Penicillin and Mastitis Control.** C. S. BRYAN, Dept. of Surgery and Medicine, Michigan State College, East Lansing, Michigan. Milk Plant Monthly, *35*(2): 27–28, 65. 1946.

The author points out that mastitis can be classified as acute or chronic.

The acute mastitis may be divided into acute local mastitis and acute systemic mastitis, the former being mastitis having symptoms of swelling, pain and the production of milk of abnormal physical appearance, while the latter is characterized by a visibly sick cow, in addition to the abnormal symptoms in the udder. Chronic mastitis is usually characterized by the absence of any painful swelling in the udder and by decreased production of milk of normal physical appearance. Because of these variations of mastitis a cow may be infected for some time before the owner is aware of the presence of the disease. Udder diseases likewise may be classified as non-infectious and infectious from the standpoint of cause. The author presents in diagrammatic form the interrelationship between the cause and the effect on the cow and her udder. In determining the cause of mastitis, while physical examination of the udder and milk are valuable, bacteriological procedures must be applied to properly collected milk samples to make specific diagnosis of infection. The dairyman is urged to consult his local veterinarian early if mastitis is suspected. In the control of bovine mastitis the following items should be carried out in order:

A. Good sanitary milking procedures. (1) Arrange cattle into healthy, suspicious and infected groups, and milk the cows in that order. (2) Wipe udder one minute before milking with a clean cloth moistened in 200 p.p.m. chlorine solution. (3) Discard the foremilk into a strip cup. (4) Do not permit persons with sores on hands or ''strep'' throats to milk the cows. (5) Dip the teat cups into a pail of chlorine solution before milking each cow. (6) Milk each cow rapidly and completely at each regular milking period.

B. Good, sanitary herd management procedures. (1) Treat all udder and teat injuries promptly. (2) Stable cows in properly constructed stalls and stanchions. (3) Clean and disinfect all stalls after removal of infected cows. (4) Keep the barn dry and permit plenty of sunshine. (5) Practice good heifer calf raising thus preventing early udder injury. (6) Replace cows through (a) home raising (b) purchase of bred heifers (c) purchase of mature milking cows, testing all replacement cows before purchase.

C. Early and accurate diagnosis. (1) Careful physical examination. (2) Microscopic test.

D. Elimination by slaughter or by complete segregation. (1) No response to udder infusion treatments. (2) Confines or eliminates the spread of infection.

E. Early proper treatment. (1) By early treatment a high percentage become free from infection. (2) Udder infusions valuable only when infection is cause of mastitis.

Penicillin, an antibiotic agent obtained from cultures of the mold *Penicillium notatum*, is a very potent agent against gram-positive bacteria, which is the cause of the greater percentage of infectious mastitis. The penicillin must be administered by a veterinarian. While this product cures many cases of infectious mastitis, the responsibility for the prevention of its re-occurrence remains on the shoulders of the dairymen. G.M.T.

FEEDS AND FEEDING

248. Seasonal Variation of Carotene and Vitamin A in Butter-Fat and in Serum.

J. W. LORD, Research Department, Messrs. J. Bibby and Sons, Ltd., Liverpool. The Biochem. Jour., *39*, No. 4: 372–374. 1945.

A herd of 70 Ayrshire cattle four to five years old and at various stages of lactation was used for the investigation. The butter-fat determinations cover a period of two years, and the serum determinations a period of ten months.

Of the total vitamin A activity of the butter-fat (carotene plus vitamin A *per se*), carotene accounted for 28% during pasture feeding and 20% or less while the animals were in stalls. The average values of the total vitamin A activity in International Units per gram of fat were 31 for pasture feeding and 15 for stall feeding. The maximum was reached in May and June and the minimum during the last half of February to the first part of April.

Blood serum determinations showed a maximum carotene content of 1.35 mg. 100 ml. during pasture feeding and a minimum of 0.23 mg./100 ml. for stall feeding. The vitamin A values for the same periods were 150 and 31 International Units/100 ml. respectively.

"The results indicate that pasture-feeding even for only a few hours a day maintains high levels of carotene and vitamin A in both butterfat and serum." A.O.C.

249. Studies in the Chemistry of Grass Silage.

J. G. ARCHIBALD, Jour. of Agr. Res., *72*: 8. April 15, 1946.

Forty samples of silage, representing various crops and treatments were analyzed to determine moisture content, pH values, total nitrogen, water-soluble nitrogen, carotene, volatile bases, total acidity, amino acids, lactic acid, acetic acid (total and free) and butyric acid (total and free). Nine samples of fresh green crops were also analyzed before ensiling which corresponded in identity with certain of the silages.

Variations due to the kind of crop used were more significant than those due to the action of the preservative. Legume silages had a lower pH value and higher total acidity than silages made from grasses or small grains. Legume silages had a high lactic acid content while the others showed relatively large amounts of acetic and butyric acids. Of the preservatives used, molasses and ground wheat seem to have been the only ones at all effective.

Salt and lactic acid cultures were in many respects inferior to no preservative at all. Urea was entirely unsatisfactory for the purpose. High carotene values did not necessarily characterize silages that were of good quality in other respects. Carotene losses in some of the large silos were excessive.

Silages made from wilted alfalfa was quite different in composition from that made from the unwilted crop. Losses in total nitrogen, as indicated by determining this element in both the fresh and the dried ground samples, were variable, being much higher in the silages than in the corresponding green crops. Certain preservatives (molasses and ground wheat) tend to minimize this loss. H.P.

FOOD VALUE OF DAIRY PRODUCTS

250. The Importance of Milk in the Health of the Nation. Frederick Tisdall, Canadian Dairy and Ice Cream Jour., *25*: 3. March, 1946.

Milk and its products must be the basis of all diets if humans are to consume the amount of riboflavin and calcium needed for good growth and health, has been determined by the R.C.A.F. doctors. H.P.

251. Fluorescence Aids in Studying the Quality of Dairy Products. Jesse A. Pearce, Canadian Dairy and Ice Cream Jour., *25*: 1. January, 1946.

A laboratory photoelectric fluorometer is described and diagrammed showing its operation. The vitamin content and quality of dairy products has been determined with this apparatus. The vitamins that fluoresce are vitamin A, vitamin B_2, and vitamin K; while vitamin B_1 and vitamin D, when treated with certain chemicals can be made to fluoresce. Fluorescence analyses have been used to detect adulteration of butter with other fats and the adulteration of cream. It can also be used to detect the source of dirt or external contamination of liquid milk. Fluoresence analysis of milk powders indicate that changes in palatability values could be correlated with changes in fluorescence. Fluorescence analysis in other dairy products studied were whey and cheese. H.P.

252. Sur la Resistance des Vitamines a L'Action de la Chaleur. (On the Resistance of Vitamins to the Action of Heat.) Jean Pien. Le Lait, *24*: 231–233. 1944.

This subject is reviewed but the literature cited contains no reference more recent than 1936. O.R.I.

253. Sur la Composition et le Rendement Énergétique du Lait de Femme en Periode de Restriction. (On the Composition and Energy

Value of Woman's Milk in a Period of Restricted Food Intake.) JACQUES LAVAGNE AND SOLANGE MATHIEU. Le Lait, *23*: 229–230. 1943.

Samples were secured from each of 13 subjects on the 5th, 8th, and, 11th days and analyzed for fat, lactose, casein and albumin, dry matter, phosphorus and calcium. From these data energy values and Ca/P ratios calculated. While the results were highly variable, it is concluded that the fat content is on the average reduced, lactose remains constant, and the caloric value reduced 21%. On the other hand, the Ca/P ratios were generally increased. This was a result of the calcium content being frequently above normal while in 19 cases out of 33 the phosphorus content was inferior to normal. O.R.I.

254. Teneur du Lait de la Vache en Vitamine PP. (Vitamin PP Content of Cow's Milk.)

MADELEINE MOREL AND J. BARATTE. Le Lait, *23*: 224–226. 1943.

The nicotinamide content of a cow's milk was determined microbiologically over a period of about five weeks during the course of which the ration was varied. Results indicated that the milk usually contained about 0.2 mg. of vitamin PP per 100 ml. and was not markedly affected by changes in ration. O.R.I.

255. Ice Cream—A Nutritious and Delicious Food.

E. A. GRANT, F. F. TISDALL, E. W. MCHENRY. Canadian Dairy and Ice Cream Jour., *25*: 2. Feb., 1946.

The amounts of protein, fat, carbohydrate, calcium, vitamin A, thiamine, and riboflavin in fourteen samples of commercial ice cream from five Ontario cities have been determined representing approximately 90% of the total commercial ice cream of the Province. A summary of the results follows:

Nutrient	Range	Average
Protein (%)	4.5– 5.6	5.0
Fat (%)	9.8–10.8	10.3
Carbohydrate (%)	15.5–21.2	17.6
Calcium (mg. per 100 g.)	130–180	150
Vitamin A (I.U. per 100 g.)	400–800	520
Thiamine (mg. per 100 g.)	50– 85	65
Riboflavin (mg. per 100 g.)	200–300	240

Data is also presented giving the comparison of some nutritive elements present in 75 grams of ice cream compared with the same nutritive elements present in other desserts served by restaurants. H.P.

ICE CREAM

256. Lessons of the Past Four Years. RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc., Philadelphia. Ice Cream Trade Jour., *42*, 4: 48. April, 1946.

Reduction in truck mileage imposed by ODT orders proved that pre-war frequency of delivery service was unnecessary, wasteful and most expensive. This lesson has resulted in loading trucks to their actual capacity, covering about $\frac{1}{2}$ the pre-war mileage and still giving ample service to dealers.

Another saving has resulted from a reduction in the number of flavors of bulk ice cream carried in stock. The number was reduced from 15 in 1941 to 7 in 1946.

These 2 factors, reduced frequency of delivery and reduced number of flavors, have made a saving in delivery costs without which we never would have absorbed the constant rise in costs of dairy products and flavoring supplies, with selling prices frozen by OPA at March, 1942, level.

Other economies which the war brought about were reduced cost of cabinet service, savings in signs and outdoor advertising cost, and a reduction in personnel in the sales department. W.H.M.

257. Bulk Gallon Sales for the Home. Ice Cream Trade Jour., *42*, 3: 24. March, 1946.

The Breyer Ice Cream Company of Philadelphia, Pennsylvania, has been experimenting with a single gallon bulk package for home consumption.

The container has inside dimensions of $6\frac{3}{4}''$ diameter, $6\frac{7}{16}''$ deep, with the well-known Breyer trade mark imprinted on it, together with instructions on how to handle the ice cream. It retails for $1.75 per gallon.

Sales have been good, during November and December, when this item accounted for 5.8 per cent of the total sales in the area where it was introduced. W.H.M.

258. 1945 Gallonage Hits All Time High. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 4: 42. April, 1946.

Estimated ice cream production for 1945 was 471,265,000 gallons, compared with 445,234,000 for 1944, an increase of 5.8 per cent. The increase was most marked after the WFO–8 was revoked in September when production for Sept., Oct., Nov., and Dec. went up 37, 47.4, 49.7, and 49.3 per cent, respectively.

The ten leading states in production were Pennsylvania, New York, California, Ohio, Illinois, Texas, Michigan, Massachusetts, Indiana, and Missouri. These states produced 60 per cent of the total volume. W.H.M.

259. **A New Cellulose Gum Stabilizer for Ice Cream.** D. V. JOSEPHSON AND C. D. DAHLE, Penna. State College. Ice Cream Rev., *28*, No. 11: 32. June, 1944.

Sodium carboxymethylcellulose (C.M.C.) is a tasteless, odorless, cellulose derivative possessing good water-binding properties and other characteristics which give it promise as a new ice cream stabilizer. This article contains the original experimental results indicating its unusual performance in ice cream manufacturing. When used in concentrations of 0.15 to 0.2 per cent of the mix it imparted good texture and a "chewy" body to the finished ice cream. This amount of C.M.C. also enhanced the whipping properties of the mix, a characteristic which is extremely desirable when natural emulsifying agents are lacking, as is the case when frozen cream is used in the mix. The synthetic product is said to be harmless physiologically. J.H.E.

260. **Use of Dried Eggs in Ice Cream.** CARL FRISCHKNECHT, War Food Administration. Ice Cream Rev., *28*, No. 10: 41. May, 1944.

There has been a great expansion of egg-drying facilities during the war. There were only 16 egg-drying plants in this country in 1941 compared to 121 in 1944. A total of 7,487,000 pounds of dried eggs were produced in 1940 compared with a total of 320,742,000 pounds in 1944. Anticipating a sharp drop in overseas demand for dried eggs, the author suggests the possibility of greater use of eggs in ice cream and reviews the advantages of eggs in ice cream. J.H.E.

261. **Technical Literature of Ice Cream for 1944.** ALAN LEIGHTON, Bureau of Dairy Industry. Ice Cream Rev., *28*, No. 10: 38. May, 1944.

This is an excellent bibliography of all the technical literature on ice cream published in 1944. There are 190 references arranged according to subject matter. J.H.E.

262. **Ice Cream Manufacturing Problems.** R. J. RAMSEY, Ramsey Laboratories, Cleveland, O. Ice Cream Rev., *28*, No. 11: 38. June, 1944.

One of the most common operating problems found in ice cream plants is the difficulty caused by a collection of ice under the blades and in the heads of continuous freezers. This ice appears in the form of tiny crystals, which are gathered together by the agitation and which form a grainy or lumpy appearance as the ice cream emerges from the freezer. Low total solids, low stabilizer, mixing ice cream coming from two freezers, excessive pressure in discharge line from freezers caused by too many elbows or lines which are too long, and mechanical troubles with the freezer, are among the causes listed.

In the matter of sugar, during this period of scarcity, most plants are trying to maintain a sweetness of approximately 13.5%. It has been found that one-half of the sugar may be replaced by corn syrup. The malt syrups and dark cane syrups work very well in chocolate ice cream and may replace up to 40% of the cane sugar. J.H.E.

263. **Low Sugar Ice Cream Mix.** D. V. JOSEPHSON, Ohio State Univ. Ice Cream Rev., *28,* No. 12: 100. July, 1945.

Ice cream containing as low as 11% sucrose sugar content was judged to be palatable but it appeared somewhat "flat." Ice creams containing 12 and 13% sucrose sugar were considered quite palatable and refreshing and compared favorably with a product containing 14% sugar. J.H.E.

264. **How to Stretch Sugar.** A. J. HANTEN, Hudson Mfg. Co., Chicago, Illinois. Ice Cream Rev., *28,* No. 12: 37. July, 1945.

It is suggested that without disturbing the sugar content of an ice cream mix the milk solids not fat can be raised to about 13%. This type of mix will justify a greater overrun because of the increased food solids per gallon. Acidity control is mentioned as a way to eliminate the problem of lactose crystallization in high milk-solids-not-fat mixes. J.H.E.

265. **Refrigeration for the Ice Cream Hardening Room.** R. A. KLOKNER, The Vilter Mfg. Co. Ice Cream Rev., *29,* No. 4: 42. Nov., 1945.

This article explains a variation in arrangement of hardening room refrigeration coils. Vertical ammonia pipes spaced on 3-inch centers attached to headers at top and bottom are arranged around the walls of the room forming partitions or bays. Advantages claimed for this type of coil construction include: ease of defrosting, uniform distribution of temperature and elimination of special ceiling height. J.H.E.

266. **Penicillin Ice Cream.** ANONYMOUS. Ice Cream Rev., *29,* No. 4: 39. Nov., 1945.

Mixing penicillin with ice cream for administering the drug by mouth in the case of throat infections has been developed by the Navy. Ice cream proved to be an ideal carrier because penicillin retains its potency for a considerable period when kept in a chilled environment. Ice cream also meets test requirements, dissolves slowly and clings tenaciously to mucous membranes. J.H.E.

267. **New Plant Construction.** ANONYMOUS. Ice Cream Rev., *29,* No. 3: 44. Oct., 1945.

Floor plans are given for two new medium-sized ice cream plants that have recently been built. J.H.E.

268. Yes I Said a Billion. R. C. HIBBEN, Int. Assoc. of Ice Cream Mfgrs. Ice Cream Rev., *29*, No. 3: 42. Oct., 1945.

Ice cream consumption more than doubled in the past ten years, and prospects are exceedingly bright that it will again double in the next ten years to reach the total of one billion gallons. The article contains facts upon which the prediction is based. J.H.E.

269. Ideal Temperatures for Storing Frozen Foods. DONALD K. TRESSLER, Consultant, Westport, Conn. Ice Cream Field, *47*, No. 4: 74. 1946.

"In general it can be said that the lower the temperature of storage the longer any given food can be held without noticeable change in flavor, color and vitamin content." In most cases the above statment holds for texture as well, the author claims.

When stored at 10° to 15° F. most fish become tough and "chewy" in about 6 months, at 0° F. these changes require from 10 to 12 months and at – 10° F. they are not apparent in less than a year.

The rate of loss of vitamin C is not the same for all vegetables, but the rate of loss of palatability and green color from vegetables usually parallels the rate of loss of vitamin C, hence vitamin C content is one of the best indices of quality for frozen vegetables.

A table is included which gives the approximate storage life of quite a variety of frozen foods at – 10° F., 0° F. and + 10° F.

The importance of proper packaging is emphasized and it is concluded that "The use of air-tight impervious containers will greatly increase the 'storage life' of any product at any given temperature." W.C.C.

270. Proposed Ice Cream Standards. MARK METCALF, Ice Cream Field, *47*, No. 4: 30. 1946.

Among the proposed standards which the Food and Drug Administration has sought to have adopted are the following:

Finished ice cream should contain not less than 12% by weight of milk fat. If nuts, fruits, confectionery products, etc, are used it must contain a minimum of 10% milk fat.

Ice cream must contain not less than 1.6 pounds of total solids per gallon and weigh not less than 4.5 pounds per gallon.

Ice cream shall contain not less than 10% by weight of fruit, juice or both, and if such products are used no other natural or artificial flavor can be used which imitates the flavor of fruit or its juice.

Nut ice cream shall contain not less than 6% by weight of properly prepared nuts.

Ice cream may not contain any ingredient which imitates the flavor of milk, cream, butter or egg.

Egg solids may be used in the form of liquid, dried or frozen eggs or yolks provided they are used to 1½ to 2% of the weight of finished ice cream. (Exact percentage to be fixed after hearing and will apply to frozen custards.)

Sherbets must contain fruit ingredients from 10% to 20% by weight. (Exact percentage to be fixed following hearing.) Sherbets should contain not less than 2% fat and weigh 6 pounds per gallon.

Sweetening agents which the Food and Drug Administration would permit include sugar, dextrose, invert sugar sirup, corn sirup, maple sirup or sugar, honey, brown sugar or molasses.

Labels are required to state specifically when any artificial flavoring is used. W.C.C.

271. **Cane Sugar.** B. W. DYER AND COMPANY, Sugar Economists and Brokers, New York City. Ice Cream Field, *47*, No. 4: 22. 1946.

It is expected that there will be a 60 per cent allotment rate for sugar the third quarter of 1946. Total 1946 civilian supplies will likely be about 5.5 million tons versus 5.1 million tons in 1945.

Liberated countries of Europe—largely sugar deficit areas—are now claimants for sugar and other foods from the United Nations supplies. The Japanese occupation materially reduced sugar production in Java and it is predicted that it will likely be 1949 before Java can export in volume.

The United Kingdom which normally received the Mauritius and Natal sugars now will demand more Cuban sugar to make up this loss. At present the United States is shipping sugar to the Philippine Islands instead of receiving nearly one million tons annually from them. W.C.C.

272. **Corn Sugar.** G. B. HAMILTON, American Maize Products Co. Ice Cream Field, *47*, No. 4: 22. 1946.

It is stated that corn sweeteners will be shorter this year than any time during the war. Since 1942, when corn supplies were adequate there has been a decrease each year in the corn available for use in manufacturing sweeteners. The author considers that the critical situation which now exists is due to the following factors: (1) Worldwide grain shortage. (2) Shortage of animal feeds. (3) Large number of animals on farms and in feed lots. (4) Farmers can receive more for their corn by feeding it than by selling it. (5) Tendency for farmers to hold corn in hopes of securing high prices. (6) Order expected April 1 limiting grind for sweeteners to 85% of 1945 quota. W.C.C.

273. **Fruits–Berries.** F. J. OWENS, Brown Packing Co. Ice Cream Field, *47*, No. 4: 20. 1946.

In January 1946 an over-all picture showed the market long on frozen apples, peaches and plums and short on strawberries, raspberries and other desirable berries.

Strawberry acreage has been increasing but raspberry acreage has decreased. It is stated that the only hope dairies have of securing adequate supplies of strawberries is the removal of price ceilings by OPA.

Puerto Rico and Cuba are expected to market as much of their pineapple crop as possible on the fresh market largely because of ceiling prices on canned fruit. Hawaii will be unable to supply a normal pineapple crop for several years due to lack of plantings because of military installations also because of dry weather.

All desirable berries will be short again this year but indications are that peaches and other tree fruit will be plentiful. W.C.C.

274. Chocolate Outlook. S. K. BLUMENTHAL, Vice-President, Blumenthal Bros. Ice Cream Field, *47*, No. 4: 19. 1946.

The rationing program on chocolate and cocoa imposed during the war is credited with materially improving conditions in the industry. It is predicted that rationing will continue for another year after which it is expected that supplies will permit production according to demands. European demands will have to be met at least in part which will reduce our supplies. Furthermore it is stated that imposed ceiling prices may prevent the United States from meeting prices paid by other countries and this in turn limit local supplies. W.C.C.

275. Cabinets—Fountains. J. J. NOONAN, Fountain-Cabinet Division, Liquid Carbonic Corp. Ice Cream Field, *47*, No. 4: 17. 1946.

Lack of material, shortage of skilled manpower as well as strikes have contributed to the shortage of ice cream cabinets and equipment for the soda fountain. Data available indicate the soda fountain industry was producing about 50 per cent of normal production capacity the first quarter but expectations are that the second quarter of 1946 would reach 75 per cent of normal production capacity. Due to lack of inventories it was predicted that customers would be likely to get about 50 per cent as much soda fountain equipment as they were able to obtain in a comparable period in 1941.

The ice cream cabinet situation is very similar to that for soda fountain equipment. The electrical strike shut off the supply of fractional motors and materially delayed ice cream cabinet production since most cabinets are of the self-contained chest type. Lack of inventory and delayed production will likely keep the supply of cabinets to about half those available during a corresponding period in 1941. W.C.C.

276. The Freezer Situation. DON LIGHTNER, Creamery Package Mfg. Co. Ice Cream Field, *47*, No. 4: 16. 1946.

Ice cream freezer manufacturers are hampered in their efforts to supply orders for equipment by lack of supplies and inadequate trained personnel. Priorities have been removed, they are filling rated orders ahead of unrated or "postwar" orders and it is stated that only a small percentage of the orders can be filled by this summer.

It is suggested that ice cream manufacturers can help by projecting their requirements over a three- to five-year period; also by eliminating duplicate orders placed with more than one freezer manufacturer. W.C.C.

277. Use of Eggs in Ice Cream. Leo D. Ovson, Ovson Egg Co., Chicago. Ice Cream Field, *47*, No. 5: 66. 1946.

It is stated that originally eggs were added to ice cream mainly to improve whipping properties, whereas now the primary reason is to increase the "richness" of ice cream. Other advantages listed for eggs in ice cream are: (1) increases smoothness, (2) improves whipping properties of mixes, (3) enhances food value of ice cream, (4) makes ice cream more resistant to melting and (5) increases emulsifying action thereby improving fat dispersion.

According to the author 26 states have laws governing the quantity of egg solids in French ice cream and frozen custard. W.C.C.

MILK

278. Practical H.T.S.T. Operation Shows Ease of Control. M. Simpson, Canadian Dairy and Ice Cream Jour., *25:* 4. April, 1946.

The operator must understand his machine to secure uninterrupted pasteurization and it will be found that the highest quality of milk is necessary. The author lists the advantages of H.T.S.T. as follows: (1) less labor is required for operation; (2) filling can be started shortly after milk is received; (3) less floor space is required; (4) continuous operation; (5) better flavor and as good a cream line; (6) a completely closed system; (7) an automatic system; (8) less time required for cleaning; (9) controls assure proper pasteurization; (10) controls assure uniform pasteurization; (11) operator can't reduce holding time to suit his will. H.P.

279. Observations of Milk Can Washing by the Acid Method. L. R. Bryant. Canadian Dairy and Ice Cream Jour., *25:* 3. March, 1946.

The results are based upon comparisons made between acid washing and alkaline washing of market milk cans, on a small commercial scale, using an ordinary small rotary type can washer at the rate of about 100 cans per day.

The observations made would indicate that hydroxyacetic acid and the detergent of the sodium hydrocarbon sulfonate type, when used as an acid can-washing solution, produce a marked improvement in the sanitary condition of the cans, as judged by odor, and in the bacteriological condition based upon the types of bacteria in the washed cans. More work is needed upon the possible corrosion effect of long time use of this acid solution upon the can-washing machine itself. A bibliography on acid can washing and related subjects is presented. H.P.

280. A Selection System for Route Salesmen and Other Personnel. J. H. Duplan. Canadian Dairy and Ice Cream Jour., *25:* 1. January, 1946.

Personnel must be carefully selected to build a good staff and strengthen confidence in the dairy industry. Some of the advantages of careful selection of milk route salesman are: (1) qualified sales force; (2) reduced turnover of routemen; (3) increased consumption and sale of dairy products; (4) reduced unit of sale operating cost; (5) higher prestige for route work as a vocation; (6) increase in good will for the industry; (7) satisfaction, happiness, and success for employee and employer. A manual published by International Association of Milk Dealers contains a list of important factors to be answered by applicant. They are mental ability, personality traits, personal history, educational background, experience background, financial standing, memberships, activities, and contacts, family standing and references. H.P.

281. L'épreuve de la Réductase dans le Controle de la Quanlité des Lait Pasteurisés. (The Reductose Test in the Quality Control of Pasteurized Milk.) A. Eyrard and Mlle. S. Rey. Le Lait, *23:* 229–230. 1943.

The authors report experimental work indicating that, on the average, methylene-blue-reduction-times of less than one hour are indicative of counts approaching 3,000,000 organisms per ml. in pasteurized milk while decreasing counts (160,000 equal to 6 hours) are associated with longer reduction times. The test lacks sensitivity at these limits. It is of value however as a rough guide as to the care with which the pasteurization process has been carried out, the extent of recontamination and the conditions under which the sample has been held after pasteurization. O.R.I.

282. Evaluation of Factors in the Processing of Homogenized Milk. E. M. Giberson, Medosweet Dairies, Ind. Tacoma, Washington. Milk Plant Monthly *35*(5): 24–25, 54–56, 60. 1946.

The author outlines extensively the various factors to be considered in the homogenization of milk, giving the advantages, disadvantages, and precautions in homogenizing milk when using either holder type or high-temperature short-time pasteurization. Clarification and filtration fully discussed.

The conclusion is reached that the consumption of homogenized milk will continue to increase as people become acquainted with its merits. Homogenizing milk equipment requires a considerably larger capital investment in milk processing. The capital outlay must be sufficiently great to enable homogenization at a low cost. An adequate system will allow the processor of homogenized milk to sell a uniform-quality product at no extra cost to the consumer. G.M.T.

283. Can Milk Distribution Cost Be Lowered? ROLLAND W. BARTLETT, Professor of Agricultural Economics, University of Illinois, Urbana, Illinois. Milk Plant Monthly, *35*(4) : 24–25, 84. 1946.

The author is convinced that in the next few years low-cost high-quality homogenized milk sold through stores will be the pace setter in the field of milk distribution. Reasons are cited for this belief. Dealers with costs for receiving, processing, bottling, administrative, selling and delivery of milk to stores not greater than 2½ to 3 cents per quart, need have little fear of post-war competition. Dealers whose wholesale costs are more than 3 cents per quart should give careful consideration to methods which will bring about more efficient operation. Cost studies are presented on retail and wholesale distribution both for paper and glass containers. No relationship seems to exist between unit costs and volume. Serious consideration should be given to the lowering of marketing costs through the use of efficient plant layouts, use of modernized machinery, reduction in plant losses, introduction of more efficient selling and delivery methods and other practical efficiency methods. G.M.T.

284. The Place of the Laboratory in a Milk Sanitation Program. C. A. PERRY, Director, Laboratory State Department of Health, Maryland. Milk Plant Monthly, *35*(4) : 26, 66–68, 83. 1946.

Since the function of the laboratory in a program of milk sanitation is to provide evidence as to the chemical and bacteriological quality of the milk, the milk regulations in various municipalities will determine the types of tests to be run in the laboratory.

Attention is called to the Standard Methods of Milk Analysis, keeping pace with dairy sanitation programs. This is an extensive manual, divided into four parts, which covers the following: (a) microbiological methods for the examination of milk, cream and butter, (b) microbiological methods for the examination of frozen desserts and ingredients of frozen desserts, (c) bioassay of Vitamin D milk, and (d) chemical methods.

Special attention is given to the tests used in Maryland in the milk sanitation program. The chief functions of the State Health Department laboratories are: (1) to make highly dependable bacteria and chemical examinations, (2) to study control measures, helping to solve problems affecting quality of milk from dairy to consumer, (3) to collaborate with milk sanitarians and inspectors in facilitating a fuller understanding of laboratory methods and findings and their interpretations, and (4) to exercise general control through advice and aid to all laboratories doing routine laboratory work. G.M.T.

285. A Comparison of the Cleansing of Square and Round Milk Bottles Under Regular Commercial Conditions. T. V. ARMSTRONG AND L. H. BURGWALD, Dept. of Dairy Technology, Ohio State University. Milk Plant Monthly, *35*(3) : 30, 32, 34. 1946.

Bacteriological studies made on the cleansing of round and square milk bottles under regular commercial conditions were made. The study involved different size bottles. Data secured show that in some instances square bottles have average counts slightly lower than round bottles. In other instances lower average counts were encountered on round bottles. Transition from the use of one type of bottle to another might account for accumulated sediment, removable with difficulty, and hence poorer washing, thus involving slightly higher counts. While the number of bottles involved in this study seemed inadequate for an exhaustive bacteriological study, the results did indicate that added difficulty for washing and sterilizing of the square bottle need not present a problem. G.M.T.

286. The Control of the Quality of Milk Supplies. HERMAN N. BUNDESEN, M.D. President Board of Health, Chicago. Milk Plant Monthly, *35*(2) : 25–26. 1946.

Milk needed in the diet of all mankind must be free from communicable disease organisms as well as have proper food value. The quality of milk is appraised by health authorities according to legal requirements governing milk production in their respective communities. These requirements usually cover inspection of dairy farms and milk plants, bacterial content of the milk and the regulation of other procedures involved in milk production.

Milk quality in many areas has deteriorated during the war years. Despite this lowering of quality, milk-borne diseases have been kept at a minimum.

The assumption is that conditions with respect to dairy farm labor and supplies will soon return to normal, and that superiority of milk quality will be regained. To this end cooperation is necessary between the milk producer and the health departments. Since cooling of milk is so important in maintaining quality of milk, milk control officials yet may have to contend with unduly high bacteria counts during the summer until artificial refrigeration becomes common for cooling milk on dairy farms. Likewise, the question is raised concerning the improvement of quality of milk if the cows are milked in a separate milking parlor.

Certification of properly sanitized hauling tanks as to the date, hour and place of cleaning and personnel involved is being prescribed by the Chicago Board of Health. United efforts of milk producers and distributors in health departments are essential. G.M.T.

PHYSIOLOGY

287. **The Quantitative Determination of Thyroxine in Iodinated Casein Having Thyroidal Activity.** E. P. Reineke and C. W. Turner, Dept. of Dairy Husbandry, University of Missouri, Columbia, and George O. Kohler, R. D. Hoover, and Margaret B. Beezley, Cerophyl Laboratories, Inc., Kansas City, Missouri. Jour. Biol. Chem., *161,* No. 2: 599–611. 1945.

Iodinated proteins having a high thyroidal activity have been shown to actually contain thyroxine. Chemical methods for estimating the potency of such preparations have usually been employed. This report compares the biological assay and a chemical extraction method. The method is based upon the hydrolysis of the iodinated casein with 40 per cent barium hydroxide solution, extraction with n-butanol and determination of the iodine content of the purified extract. Correlation between the bioassay and chemical method is fair, ranging from – 25.3 per cent to + 7.8 per cent. Fifteen iodinated casein preparations showed an average thyroxine content of 2.79 per cent determined biologically and 3.04 per cent by the chemical extraction method. A.O.C.

288. **The Effect of Manganese Compounds and Certain Other Factors on the Formation of Thyroxine in Iodinated Casein.** E. P. Reineke and C. W. Turner, Dept. of Dairy Husbandry, University of Missouri, Columbia. Jour. Biol. Chem., *161,* No. 2: 613–619. 1945.

The effect of several factors such as temperature of incubation, degree of agitation and the addition of various manganese salts in the preparation of iodinated casein is discussed.

It is believed that manganese may act as a catalyst, both *in vivo* and *in vitro,* in the oxidative process of coupling two diiodotyrosine radicals and eliminating a side chain to form thyroxine. A.O.C.

MISCELLANEOUS

289. **Opportunities in Retail Trade for Service Men.** Anonymous. Ice Cream Rev., *29,* No. 4: 98. Nov., 1945.

This is a digest of a brochure recently published by Dun and Bradstreet intended to offer guidance to discharged veterans contemplating a retail enterprise. Requirements for successful management have proven to be: personal aptitude, knowledge of the line of business, selection of location and working capital. J.H.E.

290. **Container and Packages.** J. C. La Grua, National Folding Box Co. Ice Cream Field, *47*, No. 4: 19. 1946.

The demand for paper packages is greater than the supply. Lack of raw material—wood pulp and reclaimed fibrous material—is primarily responsible for this condition although no new equipment has been available since prior to the war. Normally Sweden supplied considerable wood pulp but wood is being burned in Sweden due to lack of coal. Canadian and domestic production of pulp is being hampered by a shortage of labor. Since the end of the war lack of interest in saving waste paper contributes to the lack of reclaimed paper. Decreased government uses of paper has relieved the situation somewhat. W.C.C.

291. **Dairy Plant Sanitation Must Be Thorough and Continuous.** W. E. Noyes. Canadian Dairy and Ice Cream Jour., *25,* 4. April, 1946.

The article discusses careful training of employees with the aim of showing them the value of thorough sanitation in dairy work for company success and for their own personal advancement. H.P.

292. **Practical Suggestions for Plant Sanitation.** James T. Smith, Telling-Belle Vernon Co., Cleveland, Ohio. Nat. Butter & Cheese Jour. *37,* No. 4: 40. April, 1946.

Cleaning is just as important as any dairy plant operation. It requires properly trained personnel. Careful plant layout facilitates cleaning by allowing sufficient and convenient working space. Elimination of unnecessary pumps, fittings and overhead sanitary pipelines simplifies clean-up. Adequate light and ventilation for efficient, comfortable work are essential. For highest efficiency, workmen need clean, comfortable clothing, non-slip, water-proof footwear, and locker room facilities for their personal cleanliness. W.V.P.

293. **How to Improve Coal Utilization and Boiler Efficiency.** Theodore O. Phillips, Bituminous Coal Inst., New York, N. Y. Nat. Butter & Cheese Jour., *37,* No. 4: 50. April, 1946.

Select an economical, dependable coal supply. Get equipment to use this coal effectively and operate it according to expert engineering advice so that the coal will be completely burned with minimum loss of combustible gases. Use optimum rate of feed, control the air supply and maintain a clean boiler for best utilization of coal. W.V.P.

ERRATA

Abstract #170, Vol. XXIX, No. 6, June, 1946, page A84—title should read: **Bulk Freezing for Commercial Use.**

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CONTENTS

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Diseases
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Milk
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Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

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State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

294. The Bacterial Cell: in its relation to problems of virulence, immunity and chemotherapy. RENE J. DUBOS. With an Addendum by C. F. ROBINOW. Harvard University Press, Cambridge, i–xix, 460 pp. 1945.

Dr. Dubos' presentation of the bacterial cell is an extremely interesting one. He gives briefly and matter-of-factly the physical structures, based on judicious choice from the early literature and the latest electron micrography. One is impressed with the considerable literature on the subject within the last 5 years; it is a field many of us take as little developed. The discussion of staining is particularly good, as it relates to the physicochemical basis of staining reactions. Much emphasis is given to the Gram stain, in anticipation of later reference to the serological and other biochemical differences between the Gram positive and Gram negative bacteria.

The remainder of the book deals with the physiological significance of cellular structures. Analyses by enzymatic and immunological and bacteriophage methods are cited and interpreted in terms of what they reveal about surface and internal reactive groupings within the protoplasm. Inevitably there must follow a chapter on bacterial variability, for loss of flagella, loss of capsules, loss of specific antigens come up for discussion. The opinions expressed on nontransmissible and transmissible modifications and the possible mechanism of hereditary variaiton are cautious indeed. "In the absence of some process of fusion and segregation, it is difficult to account at the present time for the observed phenomena of bacterial variation . . ." (p. 175). And yet there follows a critique of the claims of life cycles and transformation of pneumococcal types, ending with the cryptic remark: "If the transformation thus induced is described as a genetic mutation, it offers an authentic case of specific mutation brought about by a specific treatment, a feat which geneticists have vainly tried to accomplish in higher organisms" (p. 187).

The last half of the book deals with the vastly important problems of nature of virulence, immunization against bacterial infection, and the various types of mechanisms of bacteriostatic and bactericidal agents. These subjects are certainly timely in this day of new and better therapeutic agents. The many new workers in the antibiotic and general chemotherapeutic field will find the discussion most informative.

E.M.

295. Disinfection and Sterilization. E. C. McCulloch. Lee and Febiger, Philadelphia. Sec. Ed. 1945. 472 pp. Illus. $6.50.

This book represents a thorough revision of the first edition. It includes a history of our knowledge of disinfection and sterilization, and the part played by natural agencies and the defensive mechanisms of the body. The effects of the better known physical and chemical agents together with factors affecting their activity are discussed thoroughly. A chapter on the dynamics of disinfection presents a theoretical discussion as well as a report on factors affecting disinfection in general. Methods of water purification, sewage treatment and air disinfection are discussed also.

The final chapter on factors to consider in selecting a disinfectant for various specific purposes should be quite valuable for the research and practical worker alike.

Of special interest to dairy workers are the chapters on heat, including pasteurization and heating for sanitizing of food equipment, and the discussion on factors affecting activity of chlorine compounds. The sections on antibiotics and cationic germicides unfortunately do not contain much of the information that has appeared within the last few years. The section on methods of air purification is not complete.

This book should be valuable to teachers and research workers in bacteriology and related fields. It is the only available publication that combines in one volume the various phases of disinfection and sterilization. For the reader's benefit numerous references are given to original publications. P.R.E.

296. Microbes of Merit. Otto Rahn. The Jaques Cattell Press, Lancaster, Pennsylvania. 1945. 277 pages, illustrated. $4.00.

In this unusual book the author successfully explains the nature of bacteria in terms understandable to the layman and emphasizes the seldom recognized fact that harmful bacteria comprise only a small fraction of the total microbic population on this earth. The reader will be profoundly impressed by numerous interesting descriptions of the multitude of useful activities performed by microorganisms.

The author has enlivened his descriptions with numerous excellent sketches, illustrations, and photographs. A rare sense of humor throughout adds immeasurably to the discussion. This book should be of interest not only to the layman but also to the scientist, whether he be bacteriologist or otherwise. It should be of value to dairy farmers, plant operators and laboratory workers alike because of its splendid descriptions and remarkable analogies and comparisons employed in telling what bacteria are, how they live and how they affect man's activities. Its approach is of necessity somewhat elementary. Nevertheless, it is one of the most entertaining and also valuable books ever published in the field of microbiology. P.R.E.

BACTERIOLOGY

297. Sterilization of Micro-organisms. OTTO RAHN, Prof. of Bacteriology, Cornell University. Milk Plant Monthly, *34*(12): 24–26, 46, 52. 1945.

Bacteria may be sterilized by physical methods—by the application of heat or light and by chemical methods—by adding disinfectants. The higher temperatures and stronger disinfectants must be used. Heat, drying, freezing, pressure and supersonic waves all have been utilized in destroying bacteria, heat being the best method available to dairymen. Ultra violet light not only kills vegetative cells but spores as well. Of the hundreds of different disinfectants on the market only very few are used in the dairy industry. Any disinfectant must be rejected if it is (a) strongly tasting or smelling, eliminates phenol, cresol, lysol, thymol, formaldehyde, etc., (b) colored, (c) very toxic to man, (d) corrodes the metals, and (e) very expensive. Chemical disinfectants used in the dairy industry are alkalies, chlorine, weak acids and synthetic detergents. High alkalinity may have excellent sterilizing properties, but possess a tendency to corrode tin and produce milk stone. Chlorine is very effective in water or on clean equipment. Its efficiency depends largly upon the degree of cleanliness accomplished before the chlorine is applied. Acid detergents show promising results under proper conditions of use. The non-synthetic wetting agents with decreased surface tension vary in their disinfectant properties depending to some extent on the pH of the solution used. The author believes that the chemical sterilization of dairy equipment is more promising than physical methods. However, the ideal disinfectant, one which can be used for all purposes and applied under all circumstances, has not as yet been found. More data on two new promising methods—the use of weak acids and synthetic detergents—should be obtained. G.M.T.

BUTTER

298. Many Factors Affect the Total Loss of Fat in Buttermilk. L. C. THOMSEN. Canad. Dairy and Ice Cream Jour., *23*: 8. Aug., 1944.

The factors affecting the fat loss in buttermilk are: (1) the amount of dilution of the cream or buttermilk with rinse water in the plant; (2) the richness of the cream; (3) the handling of the cream prior to churning; (4) pasteurizing methods; (5) the condition of the cream before and during pasteurizing; (6) the fullness of the churn; (7) the churning temperature and the temperature the cream was held prior to churning; (8) season of the year, feed of the cows, breed of the cows and period of lactation; (9) the churn may be of minor importance. The four tests used in testing buttermilk are: (a) the Mojonnier or ether extract; (b) the American Association; (c) the Minnesota; (d) the "regular" Babcock. H.P.

299. Standardizing the pH of Butter with Carbonate Neutralizers. W. L. DUNKLEY AND F. W. WOOD. Canad. Dairy and Ice Cream Jour., *24:* 9. Sept., 1945.

It is recommended that: (1) The neutralizing process be carried out accurately and consistently; (2) the reduction in acidity be calculated to such a value that the pH of the butter serum will be approximately 7.0; (3) the pH of the butter should be checked from time to time; (4) the pH of the butter serum be within the range 6.7 to 7.2 for best flavor and keeping quality. H.P.

300. The Flavor of Butter When Manufactured from Rancid Cream. W. L. DUNKLEY AND F. W. WOOD. Canad. Dairy and Ice Cream Jour., *24:* 7. July, 1945.

The authors believe that it is mainly the capric and lauric acids and not butyric, caproic and caprylic acids that are responsible for the bitter taste of rancid products. Neutralizing rancid cream for acidity will improve the quality of the butter, but cream should be neutralized to a titratable acidity of 0.12 or less. Other foreign flavors tend to become more evident in the butter when neutralized to a low acidity. H.P.

301. The Effect of Rusty Cream Cans Upon the Quality of Butter. F. W. HAMILTON. Canad. Dairy and Ice Cream Jour., *24:* 6. June, 1945.

Cream exposed to iron is a factor influencing the development of metallic and other off flavors in butter. With an increase of the iron surface exposed to the cream, the quality of the butter progressively depreciates. A slight increase in acidity was found in cream stored in rusty cans. Cream stored in cans that are rusty and exposes the iron in the cans is definitely deteriorated in proportion to the amount of iron exposed. H.P.

302. Bacteriology in Relation to Reworking and Printing Butter. E. G. GOOD. Canad. Dairy and Ice Cream Jour., *24:* 3. March, 1945.

All equipment used in reworking and printing butter should be thoroughly clean and sterile. Reworking (1) disturbs the structure of butter; (2) allows closer contact between microorganisms and food supply; and (3) stimulates or speeds up bacterial growth which may result in defects of a bacteriological nature. Reworking causes an enlargement of the water droplets in the butter, giving bacteria a better chance to develop. H.P.

303. Some Factors Affecting the Flavor Quality of Butter. A. H. WHITE. Canad. Dairy and Ice Cream Jour., *23:* 7. July, 1944.

The desirable characteristics of butter are good flavor, firm body, waxy texture, proper incorporation of moisture, even color and salting. The factors affecting flavor quality are: (1) cream quality; (2) enzyme action;

(3) the effect of bacterial activity; (4) chemical changes especially oxidation. The important factors involved in oxidation changes in the butterfat are as follows: (1) excessive acidity in the butter; (2) metallic contamination of heavy metals such as copper and iron; (3) exposure to light; and (4) temperature of storage. The surface deterioration of 45 commercial butters has been determined. The total bacteria counts by the plate method ranged from less than 5,000 to over 2,000,000 per c.c. for both the surface and interior of the butter samples. In general, the bacteria counts were higher on the surface than in the interior of the same sample. There seemed to be close correlation between bacterial numbers and the particular defect appearing on the butter surface, or with the original score of the butter. The copper content of butters which had developed surface defects ranged from .07 to 0.8 p.p.m., while for the butters which had maintained flavor during storage the copper contents varied from 0.09 to 0.16 p.p.m. Iron may also be an important factor in the development of fat oxidation defects. Butters that were degraded for surface defects, the pH values ranged from 5.56 to 7.56, while the pH values of the butters maintaining quality during storage were 5.94 to 7.66. The acidity of cream in relation to butter quality is becoming increasingly evident. Neutralization of cream is one of the most important operations in the creamery. Metallic contamination from bare copper equipment and exposure of butter to light are sources of trouble.

H.P.

304. Treatment of Liners as Related to Shrinkage of Butter in Fibre Boxes. R. W. BROWN AND T. L. FORSTER. Canad. Dairy and Ice Cream Jour., *23:* 10. Oct., 1944.

In a comparison of dry, 12% brine, and saturated brine treatment of liners for fibre butter boxes it was found that 12% brine solution allowed the least shrinkage, while dry liners permitted the largest shrinkage.

H.P.

305. Testing for Yeasts and Molds in Butter Encourages Sanitation. A. G. LEGGATT. Canad. Dairy and Ice Cream Jour., *24:* 1. Feb., 1945.

The presence of yeasts and molds in butter indicates a need for improvement in sanitary conditions. Yeasts and molds must enter the butter after pasteurization from imperfectly cleaned equipment. H.P.

306. Overrun and Moisture Control in Canadian Creamery Butter. G. E. TURNER AND V. E. GRAHAM. Canad. Dairy and Ice Cream Jour., *24:* 8. Aug., 1945.

The most important sources of the losses which tend to reduce factory overrun are: (1) inaccurate weighing; (2) inaccurate testing; (3) fat used in sampling and setting; (4) incomplete drainage of cans; (5) leaks in pipe lines, valves, churns, etc.; (6) accidental spilling; (7) fat losses in buttermilk; (8) remnants of butter left in the churn; (9) butter soiled in packing; (10) shrinkage allowance. Churn overrun may be reduced by lack of attention to some of the points listed above, especially 5, 6, 7 and 8. To secure proper moisture and salt content of the finished butter five requirements must be met; (1) a churn that will produce butter of uniform composition throughout; (2) the buttermaker must know exactly how much fat there is in each churning; (3) accurate moisture tests; (4) the physical condition of the butter must be such that it can be worked to the desired extent without harm to its texture; (5) an accurate method of determining the amount of water to add. The amount of salt added to butter depends upon (1) percentage of salt desired; (2) the texture of the butter Only 0.1 percent of the salt added is normally lost during the working process. Tables are given that (1) estimate the amount of salt to add to butter and (2) estimate the water to add to butter. H.P.

307. Control of Leakiness in Butter Gives More Profitable Returns. G. H. Wilster. Canad. Dairy and Ice Cream Jour., *23:* 9. Sept., 1944.

Control of leakiness in butter is essential for the following reasons: (1) economic loss either to the creamery or to the buyer and distributor on account of short weight; (2) it is necessary to compensate for loss in weight of the printed leaky butter by printing overweight units; (3) with dry wraps, leaky butter causes an unsightly wrinkled appearance of the parchment; (4) leakiness favors the growth of molds and bacteria; (5) restaurant operators and housewives object to leaky butter because they think the butter contains an excessive amount of moisture; (6) when leaky butter is exposed to air, evaporation of the moisture takes place, leaving white salt crystals; (7) the butter has a briny, harsh taste; (8) when leaky butter is spread on bread or toast, brine may spurt out to the surprise and irritation of the customer. Butter for printing should have a waxy body—not too hard and possess a well knit texture. Moisture must be thoroughly incorporated to lower shrinkage in weight. H.P.

308. New Concept About Butter Revealed by Research. W. R. Bloor, Univ. of Rochester, Rochester, New York. Certified Milk, *20*, No. 234: 5. Oct., 1945.

Feeding studies with rats have shown that the fatty acids of butterfat, which have special growth promoting values, are found in the liquid or unsaturated fractions. Weight gains similar to those induced by whole butterfat were observed where the liquid fatty acids were fed. The fatty acids of the volatile group were considerably less effective than the liquid acids in promoting growth. The relationship between body growth and the vitamin A content of the liver was also observed. The animals which had received the liquid fatty acids grew best and stored about twice as much vitamin A in their livers as did the animals which had received the solid fatty acids. Those animals which had been fed the volatile acid not only made the poorest growth, but also stored the least amount of vitamin A. This preliminary research report indicates definitely that the chemical composition of a food fat helps to determine the nutritive efficiency of other foods. It is highly probable, therefore, that some fats are more efficient than others in so far as their influence on utilization of the carotene of a mixed diet is concerned. W.S.M.

309. Estimation of Salt in Butter and New Cheese by a Mercurimetric Method. DR. W. S. ARBUCKLE, Texas Agr. Expt. Station, College Station. Natl. Butter and Cheese Jour., *37*, No. 5: 41. May, 1946.

The reagents used are: (a) 0.1711 N mercuric nitrate solution which contains 40 ml. of 2 N nitric acid per liter; (b) s-Diphenylcarbazone (Eastman 4459) indicator made with 100 mg. in 100 ml. of neutral ethyl alcohol (keep cold and in dark); and (c) 0.1 N sodium chloride to standardize the mercuric nitrate solution.

To estimate salt in butter, use the residue of the 10 gm. sample of butter remaining after moisture and fat determination by the Kohman method. Rinse the dry residue into a 250 ml. volumetric flask with three separate 30 ml. portions of distilled water. Cool rinsings and dilute to 250 ml. Pipet 25 ml. into 125 ml. Erlenmeyer flask. Add 0.6 ml. of indicator; titrate slowly with mercuric nitrate until one drop gives a pale violet. The mls. of 0.1711 N mercuric nitrate solution equal the percentage of salt.

To estimate salt in new cheese and cottage cheese, prepare sample by grinding it to a homogeneous consistency. Weigh 10 gm. into 400 ml. beaker. Add 250 ml. distilled water and heat to 150°–160° F. Mix thoroughly and cool to room temperature. Remove a 25 ml. portion of the clear solution, place it in a 125 ml. Erlenmeyer flask and titrate as for butter. The mls. of 0.1711 N mercuric nitrate used equal the percentage of salt in the sample.

This method is delicate, gives accurate results and has a sharp, permanent end point as compared to titration with silver nitrate and potassium chromate as indicator. The method is not adaptable to procedures requiring digestion of the sample. W.V.P.

BREEDING

310. Fertility and "Pregnancy Percentage." (Translated title) BERGE, S. NORDISK Jordbrugsforskning, pp. 214–224. 1942.

A discussion of various bases for computing conception rates; *i.e.* per year, per animal bred, per service, per fertile female, etc. The data were from the herd of the Norwegian Agricultural College. They include 869 cows mated a total of 6458 times. The cows were born in the years from 1885 to 1937. Therefore the possibility of some time trends in management and other conditions is present. In the whole period the percentages of cows remaining not pregnant after various numbers of services were: 38.3 after one service; 20.3 after two services; 13.7 after three services; and 10.7 after four services. Corresponding percentages for cows born in 1931 to 1937 when the health conditions were better, were 29.4%, 12.1%, 7.9%, and 5.7%. There was little difference between cows and heifers. It is recommended that cows be mated four times before they are considered barren, unless they also show other symptoms of barrenness. The repeatability of conception rates at different calvings of the same cow was .22 ± .05. This is interpreted as measuring the importance of genetic differences between cows in causing differences in their conception rates but it may have included something from time trends in environmental conditions. J.L.L.

CHEESE

311. Should Cheese be Priced on a Solids and Butterfat Basis? ARTHUR B. EREKSON, Lakeshire-Marty Co., Plymouth, Wis. Natl. Butter and Cheese Jour., *37*, No. 7: 41. July, 1946.

Wisconsin cheese manufacturers claim they cannot compete with manufacturers in states where standardization of milk for cheese is permitted. Three methods are described for calculating the value of cheese with more than the minimum fat content: (1) The value of the extra fat in a pound of high-fat cheese is determined by multiplying the amount of fat by the value of a pound of fat in cream. From this amount is deducted the value of the decreased amount of solids-not-fat in the high-fat cheese. The difference represents the extra value of the high-fat cheese and it is added to the market price for cheese with legal minimum fat and maximum moisture. (2) The amount and value of legal-minimum-fat-cheese in a pound of high-fat cheese is calculated. To this amount is added the value of the extra fat. (3) The difference in price between a pound of cheese solids and a pound of fat in cream is calculated. The amount of the extra fat multiplied by this difference determines the extra value to be added to the market price of cheese with the legal minimum of fat and maximum moisture. Three tables are given to illustrate methods of calculation and results.

In Wisconsin, where the amounts of extra fat in cheese are not as great as in some other areas, the gains accruing from pricing cheese on a fat-and-solids basis might not be great enough to offset the costs of analyses and computations; gains from standardizing would not be appreciable. But problems of merchandising might make it very difficult to pass on to the consumer the increase in price representing the extra value of high-fat cheese. These facts must be carefully considered before decisions are reached. W.V.P.

312. Should Cheese be Priced on a Solids and Butterfat Basis? W. V. Price, University of Wisconsin, Madison. Natl. Butter and Cheese Jour., *37*, No. 7: 40. July, 1946.

Cheese made from whole milk contains more than the legal minimum amount of fat. Such high-fat cheese might be paid for on the basis of its fat and solids-not-fat content when standardization of the milk is not permitted or is not desirable. The law commonly requires that the dry matter of Cheddar cheese be half fat and half solids-not-fat. Any fat in excess of this "50–50" dry matter is extra; it should be paid for according to its market value in butter or in other competitive outlets. The value of "50–50" dry matter is the price per pound of cheese divided by 0.61 (the weight of dry matter in a pound of cheese with minimum dry matter content). The value of the extra fat per pound of cheese plus the value of the "50–50" solids in a pound of cheese gives the value of the cheese. Tables and a scheme of calculations are given to simplify these calculations.

The consumer, who probably cannot tell the difference between cheeses made from whole- or standardized-milk, probably would not pay a premium for the extra fat in whole milk cheese. Manufacturers of process cheese need high-fat cheese in order to produce a finished product with 50% fat in the dry matter. It is reasonable for cheese manufacturers to expect a greater return for cheese with extra fat if they are not permitted to make the most efficient use of the milk fat by standardization. W.V.P.

313. The Value of Whey as a Food for Livestock. W. V. Price, G. Bohstedt, and I. W. Rupel. Canad. Dairy and Ice Cream Jour., *24:* 9. Sept., 1945.

Whey contains 6–7% dry matter that is of high nutritional value. The whey proteins are of the highest quality and the most easily digested. The milk sugar is a carbohydrate and has nutritive values that other sugars lack. The minerals of whey are easily assimilated. The vitamines A, B, B_2, C, found in whey are important in good nutrition. Whey makes a good feed for livestock like pigs and calves. Whey can be used as a substitute for mineral acid or molasses in making grass silage. H.P.

314. Manufacturing Cheddar Cheese From Pasteurized Milk. G. H. Wilster. Canad. Dairy and Ice Cream Jour., *23:* 10. Oct., 1944.

Many states have adopted and other states are considering adopting compulsary pasteurization of cheese-milk as a health safeguard. The advantages of pasteurization for cheese are: (1) pathogenic bacteria, if present, are destroyed by pasteurization; (2) gas-producing bacteria and other undesirable bacteria are either destroyed or greatly reduced in number; (3) there is easier control of the manufacturing procedure; (4) the cured cheese is generally of better flavor; (5) the cheese is uniform from day to day; (6) the cheese can be ripened at a higher temperature; (7) the yield is increased; (8) better quality reduces financial loss in storage; (9) more premium grade cheese is made scoring 92 to 93.

The objections to pasteurization are: (1) the cost of manufacture is increased 0.2 to 0.3 cents a pound of cheese; (2) pasteurized milk cheese ripens slower so it must be held longer; (3) the sharp flavor does not develop. H.P.

315. Considerations Governing Cooking Temperatures for Cheddar Cheese. W. H. SPROULE. Canad. Dairy and Ice Cream Jour., *24:* 1. Jan., 1945.

The cooking temperature of cheddar cheese is governed by: (1) rate at which starter is used; (2) length of milk ripening time; (3) fineness of cutting; (4) method of applying heat; (5) length of time curds are in the whey; (6) amount of agitation; (7) running acidity; (8) amount of stirring given the curd; (9) rate at which salt is applied; and (10) temperature of storage during early ripening. H.P.

316. Determination of Extraneous Matter in Cheddar Cheese. E. G. HOOD AND W. H. SPROULE. Canad. Dairy and Ice Cream Jour., *23:* 6. June, 1944.

The reagent used in the test is a 10 per cent solution of sodium citrate, filtered, made by adding 100 grams of citrate to 900 ml. of distilled water. The determination is as follows: (1) weigh 227 grams (8 oz.) of cheddar cheese curd into a clean Waring Blendor jar and add approximately 500 ml. of 10 per cent sodium citrate solution at a temperature of 45° C. Disintegrate the mixture until homogenous consistency is obtained (30 sec. or more). Transfer contents to a 2 litre white enamel measuring graduate used for heating and agitating the sample. Rinse the Blendor jar twice with additional 250 ml. and 100 ml. portions of citrate and add to the cheese citrate mixture; (2) place measuring graduate on hot plate, immerse stirrer, agitate and heat to 65° to 70° C. and continue stirring after moisture is up to temperature; (3) remove and wash off stirrer with hot distilled water catching the rinsing in the measuring graduate; (4) apply suction and transfer the mixture to the metal filter funnel; (5) wash out measuring graduate with several rinsings of hot distilled water and add to funnel; (6) wash down filter funnel when empty; (7) remove disc and heat dry in a 37° C. incubator; (8) mount discs with cellophane covering on individual cards and grade clean, fairly clean, dirty or very dirty. H.P.

317. The pH of Cheese and Its Relation to Quality. O. R. IRVINE. Canad. Dairy and Ice Cream Jour., *23:* 7. July, 1944.

The expression of acidity in terms of pH is used more and more in the cheddar cheese industry. Compact, easily operated instruments have been designed that give reliable results anywhere. Its relation to acidity in a batch of cheddar from the time the starter is added until the curd is salted shows close correlation. The discussion is presented to acquaint cheesemakers with a method which is at present finding wide application in food research work and it is at present not advocated that pH determinations be used in place of titratable acidity values as a guide in cheesemaking. H.P.

318. The Occurrence and Survival of *Brucella Abortus* in Cheddar and Limburger Cheese. A. C. DAHLBERG, Cornell University, Ithaca, N. Y. Natl. Butter & Cheese Jour., *37,* No. 5: 43. June, 1946.

See H. L. Gilman, A. C. Dahlberg and J. C. Marquardt. *The Occurrence and Survival of Brucella Abortus in Cheddar and Limburger Cheese,* JOURNAL OF DAIRY SCIENCE *29,* No. 2: 71. February, 1946. W.V.P.

319. Cheese as the Cause of Epidemics. F. W. FABIAN, Dept. of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan. Jour. Milk Technol., *9,* No. 3, 129–143. May–June, 1946.

Many cheese borne epidemics of disease have been reported. *Eberthella typhosa,* certain species of the Salmonella group and *Brucella meletensis* constitute the main group of organisms associated with many of the epidemics. *Clostridium botulinum* has been reported in a few instances. No cases of undulant fever caused by *Brucella abortus* or septic sore throat streptococci have been contracted as the result of eating cheese, although the raw milk may contain these organisms prior to the cheese making.

The survival of pathogenic bacteria in cheese varies over a wide period of time. In view of the experimental evidence, it would appear that the 60 day holding period is too short a time. It was suggested that a 90 day holding period should be the minimum time required to give the cheese an opportunity to ripen and thus destroy or attenuate the pathogenic bacteria that may be present. Pasteurization of the milk for cheese making was recommended. It was assumed that pathogenic bacteria in the cheese would die off more rapidly if the cheese was ripened at 60° F. instead of 40° F. or lower and thereby shorten the ripening period.

A combination of pasteurization and a 90 day holding period would be desirable and would produce a safe as well as a mature cheese. H.H.W.

CHEMISTRY

320. Chemistry of Butterfat Shows Many Practical Applications. W. J. Wiley. Canad. Dairy and Ice Cream Jour., *23:* 6. June, 1944.

Composed of soft and harder fats, butterfat requires special treatment according to the percentage of fats contained, and in summer with the extra percentage of softer fats, shock chilling of cream after pasteurization gives a better quality in texture and flavor to the finished butter. Fast cooling causes the absorption of the soft fats by the harder ones. Tropical spread is made by rapidly cooling molten fat to a temperature at least 20° F. below its softening point and immediately tinned. Formation of the mass of tiny crystals is sufficiently delayed to enable it to flow into the tins where it sets to a smooth spreadable solid. In order for a spread to be used as a substitute for butter: (1) it should look and taste as much like butter as possible and should possess the nutritive value of butter; (2) it should retain normal firmness and spreadability at higher temperatures; (3) it should not be subject to rapid flavor deterioration at high temperatures. H.P.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

321. Antioxidants in the Manufacture and Storage of Dry Whole Milk. S. T. Coulter. Canad. Dairy and Ice Cream Jour., *23:* 10. Oct., 1944.

The effectiveness of various antioxidants in dry whole milk based on the published experimental work are as follows: (1) two per cent oat flour delays onset of tallowiness; (2) hydroquinone in amounts equivalent to 0.01% of the weight of the milk solids is effective; (3) wheat germ oil and wheat germ oil plus citric acid are effective; (4) ascorbic acid at the rate of about 0.1% of the weight of the milk solids retards oxidation; (5) gum guaiac and nordihydroguaiarctic acid are effective but may cause fruity flavor development. H.P.

322. Fresh Liquid Buttermilk Valuable in Making Good Bread. R. W. Brown. Canad. Dairy and Ice Cream Jour., *23:* 7. July, 1944.

Experimental work in the laboratory and practical tests by bakers show that an excellent quality of bread can be made with the use of liquid buttermilk instead of the customary milk powders. H.P.

323. Dried Milk Powder II. Factors Affecting the Sorption of Carbon Dioxide. Jesse A. Pearce, Natl. Res. Laboratories, Ottawa. Canad. Jour. Res., *23,* No. 6: Sec. F: 327. 1945.

Sorption of carbon dioxide by fresh, spray-dried powder in a closed system at 35° C. and at approximately 74 cm. of mercury was observed to be greater than 0.4 c.c. per gram after 150 hours while only 0.012 c.c. of nitrogen was absorbed per gram after 70 hours. A straight line relationship between the logarithm of the amount of gas absorbed and the logarithm of time was demonstrated. Sorption curves for whole milk powder were higher than for skim powders. Great variation was observed in the sorption behaviour of powders from different plants and in powders produced at different plants and in powders produced at different times in the same plant. Temperature differences within the range of 25° to 40° C. had no effect on sorption. Palatability and the gas absorbing properties of a powder were shown to be correlated. (From author's abstract.) O.R.I.

324. Dried Milk Powder III. The Effect of Light on Keeping Quality. JESSE A. PEARCE AND W. A. BRYCE, Natl. Res. Laboratories, Ottawa. Canad. Jour. Res., *23*, No. 6: Sec. F: 334. 1945.

Exposure of both whole and skim milk to sunlight caused more rapid deterioration in quality than occurred in the dark. Ultraviolet with a principal wave-length of 3800Å accelerated the deterioration in whole milk powder stored at 38° C. but had no significant effect on skim milk powders; the effect of this light was less pronounced than that produced by sunlight. Storage of samples at 38° C. under different light intensities (produced by incandescent lamp) indicated that the difference between ultraviolet and sunlight were the result of the difference of total energy of light falling upon the sample, rather than the difference of wave-length of the activating light. (From author's abstract.) O.R.I.

325. Dried Milk Powder IV. The Effect of Storage Temperature, Moisture Content and Plant Source on the Keeping Quality of Milk Powders of Different Fat Levels. W. A. BRYCE AND J. A. PEARCE, Natl. Res. Laboratories, Ottawa. Canad. Jour. Res., *24*, No. 1: Sec. F: 61. 1946.

Milk powders with fat contents of 1, 26, 28, and 30 per cent from two plants were tempered to moisture contents of 2, 3, and 5 per cent and stored for periods up to 16 weeks at temperatures of from 40° to 140° F. Palatability deteriorated in the whole milk powders stored at 60° F. or higher, although the product from two plants varied somewhat. Palatability of skim-milk powders increased greatly during the early part of storage but later decreased when held at higher temperatures. In general a moisture content of 3 per cent was preferable for both whole and skim-milk powders. (From authors' abstract.) O.R.I.

326. Dried Milk Powder V. The Photolysis of Riboflavin in Milk Powder. W. A. BRYCE, Nat. Res. Laboratories, Ottawa, Canada. Canad. Jour. Res., *24*, No. 2: Sec. F: 123. 1946.

Exposure of milk powders to sunlight resulted in a much greater destruc-

tion of riboflavin than did exposure to ultraviolet light in the range 3200–4200Å. The rate of photolysis was greater for skim-milk powders than for whole milk powders. Increased intensities of visible light accelerated riboflavin destruction. In the spectral region of 4200 to 5600Å the band causing the greatest destruction in liquid skim milk had a principal wave-length of 4450Å, which corresponded to a maximum in the absorption spectrum of riboflavin. The rate of photolysis of riboflavin was a function of both wave-length and intensity of impinging energy. (From author's abstract.)
O.R.I.

327. The Effect of Wheat Germ Oil Antioxidants and Natural Reducing Substances on the Stability of Whole Milk Powder.

R. A. Chapman and W. D. McFarlane, Macdonald College, Quebec. Canad. Jour. Res., *24*, No. 1: Sec. F: 47. 1946.

Storage trials have been conducted on a large number of roller and spray dried whole milk powders. Accelerated tests at 65° C. were found to give an accurate indication of the relative keeping qualities where the latter were assessed by a peroxide test. Wheat germ oil antioxidants were found effective in inhibiting deterioration due to copper. Reducing substances that develop in milk powders during storage in moist atmosphere or at elevated temperatures are strong antioxidants and may offset the effect of added antioxidants. The riboflavin content of several powders with a high concentration of reducing groups decreased appreciably during storage. (From author's abstract.) O.R.I.

328. The Keeping Quality of Dehydrated Mixtures of Egg and Milk.

Jesse A. Pearce, Natl. Res. Laboratories, Ottawa. Canad. Jour. Res., *24*, No. 1: Sec. F: 70. 1946.

The storage life of a dehydrated mixture of egg and milk, when assessed by both palatability and fluorescence measurements, was shorter than the life of milk powder of similar protein, fat and carbohydrate content. Increased quantities of egg in the mixture decreased the quality of the mixture both initially and after 16 weeks storage. Those effects were noticeable at all temperatures between 40° and 140° F. but most marked above 80° F. After 16 weeks at 80° F., material packed under carbon dioxide usually had better palatability than air-packed products. The effect of added sugar was most noticeable at 120° and 140° F. Lactose had a slightly beneficial effect; sucrose was more effective.. (From author's abstract.) O.R.I.

329. Control of Composition and Quality of Condensed Milk.

Dr. O. F. Hunziker, La Grange, Ill. Natl. Butter and Cheese Jour., *37*, No. 6: 92. June, 1946.

Control of composition depends in part on concentration of the batch.

This can be best accomplished in practice by using the Baume scale. Formulas necessary for general practice are:

(1) Specific gravity of sweetened condensed $= \dfrac{100}{\dfrac{\%\text{ Fat}}{\text{Sp. Gr.}} + \dfrac{\%\text{ SNF}}{\text{Sp. Gr.}} + \dfrac{\%\text{ Sugar}}{\text{Sp. Gr.}} + \dfrac{\%\text{ Water}}{\text{Sp. Gr.}}}$

(2) Degrees Baume at 60° F. $= 145 - \dfrac{145}{\text{Sp. Gr.}}$

In equation (1) Sp. Gr. is the specific gravity of the constituent in that fraction. Thus: $\dfrac{\%\text{ Fat}}{\text{Sp. Gr.}}$ equals $\dfrac{\%\text{ Fat}}{0.93}$. The specific gravity of SNF is variable but approximates 1.608; that of sugar is 1.589; and that of water is taken as 1. In equation (2) Sp. Gr. stands for specific gravity of the finished product. These formulas can be used for either sweetened or unsweetened condensed.

Control of quality requires fresh, clean milk of good flavor, satisfactory acidity and methylene blue tests. Stainless steel equipment and vacuum pan sanitation are essential. These factors determine the keeping quality of frozen or non-frozen unsweetened condensed. Prolonged freezing of condensed milk destabilizes the proteins and the proteins of superheated condensed are the most susceptible. At 0° F., three months of storage does not damage plain condensed skim milk but 1 month will affect the superheated product. Sweetened condensed commands a good market here and abroad and since it is held before use its quality must be carefully controlled. Forewarming all milk to 170° F. for 10 minutes is necessary to prevent rancidity and mold buttons. Control of mold buttons depends also upon plant sanitation and storage of the product at 60° F. or below. W.V.P.

DISEASE

330. Resazurin and Methylene Blue Tests Influenced by Udder Cells.

S. B. Thomas and D. A. Bowie. Canad. Dairy and Ice Cream Jour., *23:* 7. July, 1944.

In order to obtain a "general picture" of the frequency distribution of udder cells in market milk, a series of 4,918 bulk herd samples were examined regularly, during the course of three years. The results show that the majority of samples (45%) had a cellular content varying between 250,000 and 750,000. Approximately 25% had less than 250.000 and 8% had a count above 1½ million. The results show that cellular reducing activity had quite a marked effect on the results of the prescribed methylene blue test. Eight per cent of the samples having a cellular content of 750,001 to 1,500,000 reduced methylene blue within 5½ hours; 25% of the heavily infected mastitis and late lactation milk reduced methylene blue within 5½ hours;

66% of the very abnormal milk samples (over 3,000,000 cells) were reduced within 5½ hours.

No sample of late lactation milk and mastitis milk, of low bacterial content and normal in appearance, were reduced below disc 4 on the 10-minute resazurin test. A number of samples with high cellular contents were reduced to disc 4. Milk heavily infected with mastitis or containing a high proportion of end of lactation milk (leucocyte content over 1½ million) was generally detected by the routine resazurin test; 65% being degraded to catgory B and 10% to category C. Twenty-five per cent of these samples reduced methylene blue within 5½ hours. H.P.

331. The Differential Diagnosis of Bovine Brucellosis From the Bactercidal Action of Blood Plasma. I. FOREST HUDDLESON, EVELYN WOOD AND AUDREY CRESSMAN, Michigan State College, East Lansing, Michigan. Certified Milk, *20*, No. 236: 10. December, 1945.

In a comprehensive vitro study of the bactericidal and growth-inhibiting action of bovine blood plasma for Br. abortus, sufficient differences were found in the action of plasma from infected and noninfected cows to differentiate one from the other regardless of the agglutination titers. With this observation as a basis a growth inhibition test was developed, which is described in detail. Thus far, the test has proved to be a highly accurate means of identifying both young and adult cows that are infected with Br. abortus, and whose agglutination titers range from 1:25 to 1:5000. The chief advantage of this test is that it can be easily developed into a routine laboratory procedure and by its application bring about the retention of many cattle that might otherwise be disposed of because of the possibility of infecting other animals. W.S.M.

332. Recent Developments in the Control of Mastitis. M. G. FINCHER, New York State Veterinary College, Ithaca, New York. Milk Plant Monthly, *34*(12): 40, 82. 1945.

In the control of mastitis two methods are considered, namely, recognition of infected cows for curing or eliminating them, and practice management which tends to minimize existing infection. While available chemicals and antibiotics have proven effective, only by a combination of this control procedure with management can best results be obtained. Mastitis control based on managed milking and proper housing of cows is preferable to an attempt to effect a miraculous cure in cows with badly diseased udders.

G.M.T.

FOOD VALUE OF DAIRY PRODUCTS

333. Butterfat Favors Vitamin-Producing Bacteria in Digestive Tract. E. B. HART, University of Wisconsin, Madison, Wisconsin. Certified Milk, *21*, No. 237: 5. Jan., 1946.

Ten different kinds of carbohydrate foods were fed to as many different groups of rats. Each of the ten groups was divided—part of them getting corn oil as the fatty portion of their ration and the others receiving butterfat. Some rats in each group were fed various levels of vitamin B. In almost all cases the rats getting the butterfat outgained those on corn oil. This was especially true of those on the normal vitamin level. Since butterfat itself contains only a negligible amount of vitamin B, it is concluded that butterfat in combination with most carbohydrates brings about a condition in the digestive tract which encourages growth of those bacteria which help produce B growth vitamins. An important possible implication of this study is the importance of butterfat in infant feeding. W.S.M.

MILK

334. Clarification Lessens Sediment in Homogenized Milk. I. I. PETERS AND G. M. TROUT. Canad. Dairy and Ice Cream Jour., *24:* 3. March, 1945.

Clean milk clarified and pasteurized before homogenization, removes leucocytes and other matter that forms a sediment in the bottled milk. The problem of sedimentation is aggravated by every-other-day delivery, longer home refrigerator storage and stacking of bottles horizontally during storage. Clean low-leucocyte milk may be homogenized, without clarification. Clarification may be done effectively even when the fat is in a solid state. The pasteurization temperature appears to have no significant influence on sediment formation. Sedimentation of nonclarified milk is enhanced by heat-shocking and agitation. H.P.

335. Future Operations in the Market Milk Industry. P. H. TRACY, Canad. Dairy and Ice Cream Jour., *23:* 6. June, 1944.

The writer gives in detail what we can expect in the future operations in the market milk industry. The transition from war to peace will continue the demand for fluid milk. Consumer demand will be at a high level for some time due to depleted supply of many commodities. Efficiency in the processing plant will have to be maintained as it will become increasingly difficult for the inefficient to survive because of the narrow margin of profit per unit that will exist. More milk will be sold through stores. Some high operating costs are due to: (1) plant arrangement; (2) obsolete equipment and methods; (3) excessive man hours for the amount of milk handled; (4) high fat loss; (5) high glass bottle loss; (6) returned milk; (7) delivery expense high; (8) office expense high; (9) maintaining too many grades of milk and cream; (10) special delivery; (11) not enough attention paid to personnel problems. Thirty-one factors for conditions for greater efficiency are listed. H.P.

336. Control of Mold Growth in Composite Milk Samples. J. M. FRAYER. Canad. Dairy and Ice Cream Jour., *23:* 8. Aug., 1944.

Mold growth is commonly first observed on the stopper. It may be effectively controlled by thorough cleaning and scalding the bottles and by soaking the well-cleaned stoppers overnight in a 50–50 formalin solution between each testing period. Rubber stoppers should be used since they withstand hard usage and can be sterilized. Adequate sample refrigeration is absolutely essential (50° F. or less). Covered trays or boxes, perferably of metal, easily cleaned, should be provided and thoroughly cleaned and sterilized between testing periods. Sample should be promptly refrigerated, kept cool until they are tested and held cool during the specified retest period.

Mold in composite samples (1) may test too low; and (2) are hard to pipette. Mold get into composite sample bottles (1) from the milk, (2) from the sampler's hands and clothes; (3) from the sample bottles and stoppers; (4) from the sample storage boxes; (5) from the refrigerator walls, ceilings and air. H.P.

337. An Appraisal of Inspection Problems in Sanitation. R. S. BREED. Canad. Dairy and Ice Cream Jour., *23:* 8. Aug., 1944.

The farm problems are: (1) labor shortages; (2) dirty stables; (3) dirty cows; (4) poor ventilation and light in cow barns and milk houses; (5) the presence and housing of animals other than cows in the cow barn; (6) dirty milking stools; (7) improper milk methods; (8) improper storage of milking machine and other utensils; (9) lack of suitable toilet facilities near milk house or barn; (10) wash tanks and hot water; (11) unsafe water supply; (12) lack of cooling facilities; (13) replacement of damaged or defective utensils and other equipment; (14) mastitis; (15) delay in milk deliveries; and (16) rewashing of milk cans on the farm.

The plant problems are: (1) shortage of skilled man-power; (2) inability to get new equipment; (3) inability to get repair parts for old equipment; (4) the production of quality milk under present regulations. H.P.

338. Causes of the Variations in the Percentages of Fat in Cream. R. W. BROWN. Canad. Dairy and Ice Cream Jour., *23:* 10. Oct., 1944.

Some of the causes of variations in the percentage of fat in cream are: (1) position of the cream and skimmilk screw; (2) the speed and true running of the separator; (3) the temperature of the milk; (4) the amount of milk in the separator supply tank; (5) the amount and kind of flush (whether water or skimmilk); (6) the cleanliness of the separator bowl; (7) the feed of the cows; (8) the period of lactation and (9) the speed at which the separator is turned. H.P.

339. **The Source of Acidity of Fresh Milk from Jersey Cows.** Allen D. Robinson and Herbert S. Samson, University of Manitoba, Winnipeg, Canada. Canad. Jour. Res., *24,* No. 1: Sec. B: 5. 1946.

Thirty samples of Jersey milk were analyzed for each of titratable acidity, free carbon dioxide, carbonates, citric acid and citrates, casein, lactalbumin and lactoglobulin, and inorganic phosphates. Partial correlation coefficients were calculated for titratable acidity with each of the other variables. The only significant one was that between titratable acidity and inorganic phosphates and it is concluded that inorganic phosphates are the primary cause of the acidity of fresh milk. (From author's abstract.)
O.R.I.

340. **Etude d'une Reaction Péroxydasique Appropriée pour la Detection de L'eau Oxygénée dans les Laits de Consummation.** (Study of a Peroxidase Reaction Suitable for the Detection of Hydrogen Peroxide in Market Milk.) A. Egard and R. J. Jourfret. Le Lait, *23,* No. 224–226: 141. 1943.

The detection of added hydrogen peroxide is very difficult in raw or pasteurized milk except within a short time after it has been added. A test based on the development of a blue color with guiacol and gum guiac in alcohol is described which revealed the presence of hydrogen peroxide up until approximately 7 hours for raw milk and over 30 hours for pasteurized milk. O.R.I.

341. **La Signification de la Reaction de Storch, de la Vitesse de Montee de la Creme, Ainsi que de la Determination des Phosphases Pour de Controle de la Pasteurisation du Lait.** (The Significance of the Storch Reaction and the Speed of Cream Rising Compared with the Determination of Phosphatases for the Control of Milk Pasteurization.) A. K. Van Bever and J. Straub, Amsterdam Food Products Inspection Laboratory. Le Lait, *23,* No. 224–226: 97 and No. 227–228. 1943.

The necessity for evolving accurate means of control for use with pasteurization techniques now in use is pointed out. The work of European investigators on the Storch reaction and on cream rising is reviewed. A test based on peroxidase activity of improved accuracy is described, but the test is rejected since milk flavors are affected before the test is useful and also because the addition of raw milk to pasteurized milk is not detectable in amounts of less than 10%. An accurate method of determining the rate of cream rising is described, and the effect of heat on this phenomenon discussed. Curves based on values found in the literature for destruction of tubercle bacilli, denaturation of milk proteins, phosphatase activity and cream agglutinin are treated mathematically and a formula developed showing limits of safety allowable for continuous methods of pasteurization. The authors reject the standards of Kay and insist that phosphatase activity be completely destroyed. See Enzymologia *11;* 7–8, 1943. O.R.I.

342. Milk Containers. R. B. STOLTZ, Ohio State University, Columbus, Ohio. Milk Plant Monthly, *34*(12): 49, 53. 1945.

Economy in milk distribution is in part effected by the kind of milk container used. Glass bottles average normally about 40 trips. Average bottle trippage in Columbus, Ohio is 80. Paper bottles may be more economical when glass bottles make seven trips or less. The cost of containers in glass depend upon some following factors: (1) number of trips that a bottle makes, (2) cost of bottle exchange, (3) cost of sorting bottles, (4) bottle deposit enforcement, and (5) method of handling bottles. Dealers in Columbus, Ohio using a universal bottle since 1934 have changed from the 22½ oz. 56 mm. bottle to the 17¾ oz. 48 mm. square bottle and have effected marked material savings. The inauguration of a three-day a week delivery made necessary the conservation of refrigerator space, which was accomplished in part by the use of the square bottle. It is recommended that every milk distributor ascertain his container costs by ascertaining bottle trippage. G.M.T.

PHYSIOLOGY

343. Influence of Dietary Factors and Sex on the Toxicity of Carbon Tetrachloride in Rats. PAUL GYORGY, JOSEPH SEIFTER, RUDOLPH TOMARELLI AND HARRY GOLDBLATT, School of Medicine, University of Pennsylvania, Wyeth Institute of Applied Biochemistry, Philadelphia, and School of Medicine, Western Reserve University, Cleveland. Jour. Expt. Med., *83,* No. 6: 449–462. June, 1946.

Six groups of rats fed different diets were exposed to the inhalation of 300 p.p.m. of carbon tetrachloride for 150 days. The rats maintained on a low protein diet showed greater susceptibility than animals kept on a high protein diet. Methionine can be substituted for protein (casein) in the diet satisfactorily. A high fat and low carbohydrate intake was noticeable in the rats, especially if combined with a low protein diet.

Intoxication of the rats inhaling carbon tetrachloride showed evidence of necrotizing nephrosis and certain hepatic changes such as necrosis and cirrhosis.

Dietary factors (methionine containing protein) as well as low fat intake usually prevented renal injury.

Under comparable dietary conditions, especially with high fat intake, the male rats were more susceptible to carbon tetrachloride than the female animals. H.H.W.

MISCELLANEOUS

344. **Thermometers, Their Manufacture and Industrial Usages.** E. E. R. Penfold. Canad. Dairy and Ice Cream Jour., *24:* 6. June, 1945.

Thousands of operations in the dairy industry demand that accurate temperatures be maintained and this calls for thermometers that are precision manufactured and individually tested before being placed in operation. The three thermometers that are recognized as standard and are in universal use today are the Fahrenheit, the Centigrade and the Reaumur, the first two named being most used in dairies and in dairy investigating laboratories. All glass for thermometers used in America is made by the Corning Glass Works, Corning, N. Y. Glass for thermometers must expand or contract quickly. In testing thermometers, it is important that the medium in which the testing is done is thoroughly agitated. It is also important to supply vertical agitation to prevent the hot liquid from collecting at the top. Water is the best medium for testing thermometers whenever the temperature permits. Oil may be used from 212° F. to 550° F. Sufficient time must be allowed for the thermometer to reach the temperature of the liquid. Mercury thermometers are more accurate than red spirit thermometers. Recording thermometers are used extensively in the dairy field today. H.P.

345. **At Last the Fly Has Met Its Master.** John G. Matthysso, Geigy Co., Inc., New York City. Certified Milk, *21*, No. 237: 3. Jan., 1946.

Experiments on the use of DDT were conducted at the Walker-Gordon Certified Milk Farm at Plainsboro, which comprises some 1500 cows in 33 similar barns. It was found that 21 sprayed barns became relatively fly-free at once, a reduction of 70 to 80 per cent in the fly population still being noted 20 days after application. In 30 days the comparison was outstanding, averaging from 0.1 to 0.5 flies on 10 cows in sprayed barns to 16 on 10 cows in unsprayed barns, a reduction of from 97 to 99 per cent. At 85 days there was no difference; the spray deposits were no longer effective. Another benefit from the fly control program was the absence of a single case of "pink eye" (a fly borne disease of cattle) in the milking herd. For application of the DDT to walls, ceilings, posts, etc., a power sprayer such as an orchard sprayer or power white-washing unit is recommended. A three or four nozzle broom type gun with No. 3 discs is preferable. In the latitude of New Jersey and Pennsylvania and North two sprayings a year will give effective control of flies in barns. The first application should be made when the flies first appear and the second application between July 15 and August 1. It is pointed out that DDT is not a substitute for farm sanitation and that manure disposal practices should be continued.

W.S.M.

346. Apprenticeship Training Applied to the Dairy Industry. W. F. Simon. Canad. Dairy and Ice Cream Jour., *23:* 6. June, 1944.

Indentured apprenticeship training has been in effect in Wisconsin since 1911. Skilled trades are learned by young men under an apprenticeship agreement. The agreement covers (1) the terms of training (2 years); (the first four months is a trial during which either party can void the indenture) (2) the kind of work the apprentice is to perform; (3) at least four hours weekly must be spent in school instruction regardless of the trade; (4) a wage scale must be specified for the full term of training. The author states that this is probably the best way to get qualified trained men for certain kinds of skilled trades in the dairy industry. H.P.

347. Use of New Insecticides in Dairy Plants. E. H. Fisher, University of Wisconsin, Madison. Natl. Butter and Cheese Jour., *37,* No. 7: 50. July, 1946.

Sanitation in the dairy plant must be habitual and must precede effectual use of insecticides. DDT is efficient in controlling more insects than any other conventional insecticide and remains effective longer after application. It must be applied with precautions for the safety of the user. Food products must be protected from insects affected but not promptly killed by the DDT. DDT in wettable powder sprays leaves a white residue and is useful on porous surfaces because it remains on the surface. Oil-in-water emulsions soak in somewhat on porous surfaces but not as much as solutions of DDT in deodorized kerosene or similar oil base solutions. DDT paints have not proven economical. DDT dusts can be placed in cracks. For flies DDT is used in 5% solution. Cockroaches can be treated with 10% DDT dust, 5% DDT spray, sodium fluoride dust, pyrethrum dust, Lethane A–70 dust (10%), phosphorus paste, moistened baits with toxicants like DDT or sodium fluoride, or by fumigation. Ants can be treated with DDT dust around the ant hill, by putting carbon bisulfide in the hill, and by poisoned foods containing tartar emetic, sodium arsenate, or other poisons. Cheese mites can be destroyed by fumigation with cyanide or methyl bromide. Detailed instructions and precautions for the application of these insecticides are given. W.V.P.

348. A New Germicide for the Food Industries. W. E. Botwright, Vestal Laboratories, Inc., St. Louis, Missouri. Jour. Milk Technol., *9,* No. 2: 101–106. March–April, 1946.

The problem of controlling bacteria, yeasts and molds in food and beverage industries is very important in order to prevent spoilage of the product, to prevent illness caused by microbial growth in these products and to prevent the transmission of the diseases by utensils.

Obviously, the cleansing and sanitizations of all equipment and utensils used in the processing and dispensing of food and beverage products is highly desirable. There are two procedures generally used in the sanitization of these materials: (1) heat treatment, such as water at 170° F., steam or dry heat and (2) immersion in a chemical germicide. Sanitarians are aware of the limitations and disadvantages of these methods.

Quaternary ammonium germicides are gaining in popularity in the dairy and food industries as effective germicides. Investigations show that these compounds have many advantages over the compounds now in general use. H.H.W.

349. Packaging III. Effect of Mold Growth and Ageing on the Water-Vapor Transmission of Packaging Materials. C. G. LAVERS AND W. I. ILLMAN, Natl. Res. Laboratories, Ottawa, Canada. Canad. Jour. Res., *24,* No. 2: Sec. F: 117. 1946.

Packaging materials were dusted with mold spores and stored in a cabinet at 95° F. and 95–100% relative humidity for periods of one to eight weeks. M.S.T. and M.S.A.T. "Cellophane" were attacked only slightly by mold but deterioration of the heat-sealing, moisture-proof lacquer occurred during storage under conditions suitable for optimum mold growth. Molds grew abundantly on M.S.Y.T. Cellophane. Wax-coated materials supported abundant mold growth and their water-vapor transmission values increased when wax peeled from the surface of the sheet. The transmission rate of laminated materials having metal foil as one layer was not greatly affected by mold growth or de-lamination of the layers. Abundant mold growth developed on most samples of kraft, and on glassine. Very little developed on cellulose acetate, pliofilm, or vinyl-film. (From author's abstract.) O.R.I.

350. The Newer Dairy Cleaners. H. A. TREBLER, Sealtest, Inc., Baltimore, Md. Milk Plant Monthly, *34*(2): 47–48. 1945.

In cleaning dairy equipment the availability of a sufficient, suitable water supply is very important. The function of dairy cleaners is simply to modify the water, thus improving its natural ability to dissolve protein, suspend or emulsify dirt. A good dairy cleaner whether acid, neutral or alkaline should (1) help the water dissolve milk proteins, (2) reduce interfacial tension and (3) should make possible free rinsing. Alkaline cleaners obtain good results in can washing and general cleaning when they maintain in the solution (1) a temperature over 110° F., (2) an active alkalinity of .02 to .03%, (3) a wetting agent concentration (Nacconal N R or equivalent) of .008% (80 p.p.m.) and (4) a slight surplus of calcium sequestering agent. With acid cleaner at pH of 6.5 to 6.8, calcium and magnesium precipitates will not be formed. Addition of 5% pyrophosphate results in obtaining brighter bottles with minimum carry over in the bottle washing machine. Quadrofos or Calgon and one-half wetting agent, appears to be the best cleaner for cleaning milking machines. G.M.T.

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CONTENTS

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Cheese
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Diseases
Feeds and feeding
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Milk
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ABSTRACTS OF LITERATURE

BOOK REVIEWS

351. Currents in Biochemical Research. Edited by David E. Green. 486 pages. $5.00. Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y.

"*Currents in Biochemical Research* represents an attempt by some thirty research workers to describe in as simple language as possible the important developments in their own fields and to speculate a little on the most likely paths of future progress. The aim of these essays has been to excite the imagination and to provide glimpses of some of the fascinating horizons of biochemical research."

The subjects treated and the authors of these essays are as follows: (1) The Gene and Biochemistry, G. W. Beadle; (2) Viruses, W. M. Stanley; (3) Photosynthesis and the Production of Organic Matter on Earth, H. Gaffron; (4) The Bacterial Cell, René J. Dubos; (5) The Nutrition and Biochemistry of Plants, D. R. Hoagland; (6) Biological Significance of Vitamins, C. A. Elvehjem; (7) Some Aspects of Vitamin Research, Karl Folkers; (8) Quantitative Analysis in Biochemistry, Donald D. VanSlyke; (9) Enzymic Hydrolysis and Synthesis of Peptide Bonds, Joseph S. Fruton; (10) Metabolic Process Patterns, Fritz Lipmann; (11) Biochemistry from the Standpoint of Enzymes, David E. Green; (12) Enzymic Mechanisms of Carbon Dioxide Assimilation, Severo Ochoa; (13) Hormones, B. A. Houssay; (14) Fundamentals of Oxidation and Reduction, Leonor Michaelis; (15) Mesomeric Concepts in the Biological Sciences, Herman M. Kalckar; (16) Viscometry in Biochemical Investigations, Max A. Lauffer; (17) Isotope Technique in the Study of Intermediary Metabolism, D. Rittenberg and David Shemin; (18) Mucolytic Enzymes, Karl Meyer; (19) Some Aspects of Intermediary Metabolism, Konrad Bloch; (20) The Steroid Hormones, Gregory Pincus; (21) Plant Hormones and the Analysis of Growth, Kenneth V. Thimann; (22) Chemical Mechanism of Nervous Action, David Nachmansohn; (23) Some Aspects of Biochemical Antagonism, D. W. Wolley; (24) Chemotherapy: Applied Cytochemistry, Rollin D. Hotchkiss; (25) Biochemical Aspects of Pharmacology, Arnold D. Welch and Ernest Bueding; (26) Some Biochemical Problems Posed by a Disease of Muscle, Charles L. Hoagland; (27) Physiology and Biochemistry, Surgeon Captain C. H. Best; (28) X-Ray Diffraction and the Study of Fibrous Proteins, I. Frankuchen and H. Mark; (29) Immunochemistry, Michael Heidelberger; (30) Social Aspects of Nutrition, W. H. Sebrell; (31) Organization and Support of Science in the United States, L. C. Dunn. T.S.S.

352. Annual Review of Biochemistry, Vol. XV, 1946. EDITED BY J. MURRAY LUCK. 616 pages plus author and subject indices. $5.00. Annual Reviews Inc., Stanford University P. O., California.

Again Annual Reviews Inc. brings us up to date in the biochemical field with Volume XV of Annual Review of Biochemistry. The subjects covered and the authors are as follows: (1) Biological Oxidations and Reductions, K. A. C. Elliott; (2) Non-Oxidative Enzymes, A. M. Wynne; (3) Plant Carbohydrates, S. Peat; (4) The Chemistry of the Lipids, J. B. Brown; (5) The Chemistry of the Proteins and Amino Acids, T. L. McMeekin and R. C. Warner; (6) The Chemistry of the Steroids, T. Reichstein and H. Reich; (7) Carbohydrate Metabolism, C. F. Cori and G. T. Cori; (8) Fat Metabolism, W. C. Stadie; (9) The Metabolism of Proteins and Amino Acids, D. Rittenberg and D. Shemin; (10) The Vitamins, R. A. Dutcher and N. B. Guerrant; (11) The Chemistry of the Hormones, H. Selye and H. Jensen; (12) The Biochemistry of Teeth, H. M. Leicester; (13) Growth Factors for Microorganisms, E. E. Snell; (14) Photosynthesis, C. S. French; (15) The Respiration of Plants, W. O. James; (16) The Biochemistry of Yeast, C. Neuberg; (17) Bacterial Metabolism, H. A. Barker and M. Doudoroff; (18) Immunochemistry, E. A. Kabat; Organic Insecticides, W. M. Hoskins and R. Craig; The Viruses, N. W. Pirie; Inactivation and Detoxication of Pressor Amines, W. H. Hartung.

The increasing tempo of activity in the field of biochemistry makes the Annual Review a must in the library of the teacher and research worker. The present volume maintains the high level of those that have previously appeared. T.S.S.

353. Enzymes and Their Rôle in Wheat Technology. EDITED BY J. ANSEL ANDERSON. Published for the American Association of Cereal Chemists by Interscience Publishers, Inc., New York. 1946. 371 pages, 11 chapters, indexes.

This is the first of a proposed series of monographs sponsored by the American Association of Cereal Chemists on subjects of immediate interest to cereal chemists. Enzymes play a very important part in cereal technology. This monograph is restricted principally to those of greatest interest to cereal chemists. It includes the amylases, proteases, lipases, oxidases and the fermentation enzymes. For each of these are twin chapters; the first covering a broad review of existing knowledge of the class, including both plant and animal enzymes, the second a discussion of the rôle of the particular enzymes in wheat technology. This monograph involves, therefore, a review of the knowledge extant on the properties of the enzymes, and the application of this knowledge to utilization of cereals, particularly wheat, in milling, baking and fermentation processes. The increasing diversity of processes within the dairy industry includes those in which enzymes play an important part, viz., malt products, protein derivatives, cheese, and ferment products. This book is to be highly recommended to the student, and to the dairy research laboratory for the fundamental and practical knowledge it provides. Various chapters written by cereal chemists include: an excellent review of the general chemistry of enzymes; amylases, and their applications in milling and baking technology; esterases, their rôle in milling and baking; oxidizing enzyme systems, their occurrence and effects in wheat and flour; proteases, their rôle in baking; the mechanism of alcoholic fermentation, yeast fermentation. K.G.W.

BUTTER

354. Bacteriology of Butter. VIII. Salt Distribution in Butter and Its Effect on Bacterial Growth. W. H. HOECKER AND B. W. HAMMER. Iowa State College Res. Bul. 339. 1945.

Micromethods were used to determine the moisture and salt contents of butter. It was found that the moisture and salt contents became more and more uniform as working progressed and their distribution was uniform in well-worked normal butter. Generally, in mottled butter, the light-colored portions contained the least salt and always the smallest droplets of water.

When butter was first salted and lightly worked, the water droplets in the original butter granules were free from salt. It is believed that even in well-worked butter there were some of these droplets without salt. Moisture and salt distribution may be made less uniform by printing.

When organisms causing butter spoilage were added to churned butter before working, the presence of 1.5% salt slowed down their growth and the rate of spoilage. However, when these organisms were added to the cream just before churning, the results varied. When the butter was poorly worked spoilage was often more rapid as the moisture in the original butter granules had no salt. As working progressed the salt distribution and keeping quality were improved for fewer water droplets were free of salt. A.C.D.

355. German Continuous Butter Churn. Jour. Milk Technol., *9*, No. 3: 143. May–June, 1946.

A continuous butter-making machine was captured in Germany by the Quartermaster Corps Intelligence Team. The machine was designed to produce 1500 pounds of butter per hour, and was manufactured by Roth Moelkerei Maschinenfabrik in Stuttgart.

The machine will be tested by the Research Committee of the American Butter Institute under contract with the Quartermaster Corps Food and Container Institute. Dr. H. A. Ruehe, Department of Dairy Husbandry, University of Illinois, will supervise the experiments in the Beatrice Creamery Company plant at Champaign, Illinois.

The results will be reported to the Quartermaster Corps and turned over to the Office of the Publication Board for general release to all interested persons. H.H.W.

CHEMISTRY

356. The Fatty Acids of Human Milk Fat. J. B. Brown and Betty M. Orians, Laboratory of Physiological Chemistry, Ohio State University, Columbus, Ohio. Arch. Biochem., *9,* No. 2: 201–219. 1946.

The relatively large volume of 52 liters of human milk, yielding 1300 grams of fat, was used for this investigation. The component fatty acids were separated by the ester fractionation procedure followed by low-temperature crystallization. Details of the method as well as six separate charts outlining each step are given. A comparative table of fatty acid values reported by the principle previous investigators as well as the present work is presented. The authors conclude that "no evidence could be found for the presence of more than traces of acids below C_{10}" and—"the amount of linolenic acid is negligible. The finding of dienoic acids of series above C_{18}, . . . was confirmed, and in addition evidence was presented for the presence of high molecular weight trienoic acids. This specimen of fat is much more similar to human body fat than it is to a typical milk fat." A.O.C.

357. The Distribution Pattern of Fatty Acids in Glycerides of Milk Fat. E. L. Jack, J. L. Henderson, and E. B. Hinshaw (From the Division of Dairy Industry, University of California, Davis). Jour. Biol. Chem., *162,* No. 1: 119–128. 1946.

Milk fat (neither the source nor the season of the year are given) was dissolved in Skellysolve A and fractionated by freezing out at various temperatures (for detailed description see Jour. Dairy Science, *28:* 65–78, Jan., 1945). These various fractions, as well as the methyl esters obtained from each, were analyzed. The molar percentage of the various fatty acids, which include the C_4 to C_{22} both saturated and unsaturated, are listed for each fraction and are compared with similar values on the whole fat.

Considerable discussion is devoted to the probable distribution pattern of the various fatty acids in the glycerides and evidence is given to show that it "tends more nearly to the pattern of *widest possible distribution* than to the pattern of *random distribution.*" Some interesting hypothetical patterns are compared with the experimental data. A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

358. The Effect of Fat on the Utilization of Galactose by the Albino Rat. R. P. Geyer, R. K. Boutwell, C. A. Elvehjem, and E. B. Hart, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison. Jour. Biol. Chem., *162,* No. 2: 251–259. 1946.

In previous work the Wisconsin workers reported that when whole milk was fed to rats, calves and pigs rather than skim milk as the sole diet there was less galactose excreted in the urine than on the non-fat diet. Other investigators later concluded that fat as such had no influence upon the utilization of galactose. The work reported here is a more detailed investigation relative to the influence of fat on galactose utilization when skim milk or synthetic rations containing lactose or galactose were fed to rats. It corroborates their previous work and substantiates the statement that "not only various fats such as butter fat, cocoanut oil, etc., were effective, but also that the fatty acid fraction was the active component of the fat." It was shown that only the even-numbered fatty acids having more than 10 carbon atoms could function in lowering the urinary galactose loss, and that glucose was found to be slightly effective with some of the animals. They further concluded that "Fat increased the utilization of galactose by the rat when either lactose or galactose is ingested. This phenomenon occurs on milk or synthetic type ration." A.O.C.

MILK

359. Quality Control of Milk as It Enters and Leaves the Plant. E. A. CRAWFORD, Dept. of Health, New York City. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 67. 1945.

First physical examination of milk is the odor. "Off odors" are usually due to feed, excessive bacterial content, and mastitis-infected udders. A reddish tinge in color indicates mastitis. The suction type sediment test is now preferred. The strainer dipper shows both excessive sediment and flakes. The temperature of the milk is obtained approximately by the hand on the outside of the can.

Plate counts on producers' milk are made by some plants. The direct microscopic count is probably best on milk with counts over 150,000 per ml. The Department of Health requires that plants using high-temperature short-time pasteurizers shall make weekly thermoduric counts on each source of milk supply. If three consecutive counts are high, then thermoduric counts must be made on each producer's milk. Producers' milk with thermoduric counts over 20,000 per ml. must be corrected. High thermoduric counts are generally caused by milking machines, milk stone, re-used strainer cloths, and open can seams.

Farm inspection is important. Recently cryoscopic tests on producers' milk have shown evidence of adulteration by watering in 10% of the samples.

At the raw milk shipping plant it is important to promptly cool the milk to 36° F. or below and run directly into well-cleaned and sterilized tanks which should be nearly filled with milk. A.C.D.

360. Control of Milk Watering. PAUL CORASH, Dept. of Health, New York City, 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 53. 1945.

Tests on retail milk in New York City show 0.6 to 1.8% to be sub-standard in butterfat or total solids in the past 5 years. Recently, the Department of Health began checking producers' milk for watering by determination of freezing points. Pure milk has a freezing point varying from -0.53 to -0.56° C. Night's and morning's milk for the same herd have the same freezing point. When the results of the test were to be confirmed, samples of milk were obtained from observed milkings on the farm. Tests should not be made on samples with increased acidity.

Rather striking evidence of watering milk was secured in some instances. This method of detecting watering was found to be very useful. A.C.D.

361. Water Supplies for Milk Plants and Dairy Farms. F. N. THOMSON, N. Y. State Dept. of Health, Albany, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 7, 1945.

It seems quite unnecessary to emphasize the need for an ample supply of pure, safe water for a milk plant or dairy farm. There are certain difficulties with pollution that are sometimes experienced. In appraising the source of water it is necessary to consider the surroundings, the development, and the results of laboratory tests.

Although 100 to 200 feet distance between pollution and the water source is usually ample, there are some noted exceptions. In a limestone area the sewage from a milk plant polluted a spring 1½ miles away. Sometimes leaky sewers have been found to pollute wells. Many farm wells do not have satisfactory protection against surface contamination draining back into the well.
A.C.D.

362. The Lack of Preservative Action of Surface-active Cationic Germicides in Milk. ADRIEN S. DUBOIS AND DIANA D. DIBBLEE, Onyx Oil and Chemical Co., Jersey City, N. J. Jour. Bact., *51*, No. 3: 406. March, 1946. Abs. Proc. of Local Branches.

"The bacterial count of raw milk was not affected by the presence of 1:500 to 1:25,000 dilutions of alkyldimethylbenzyl-ammonium chloride, upon incubation at either 10, 20, or 37° F. However, 1:500 and 1:1,000 dilutions of the germicide caused an immediate, appreciable reduction in the initial count of the milk. Less acid was produced in the treated milk during incubation. This was especially noticeable with the higher concentrations of the germicide.

"The lower acidity in the treated milk is assumed to be due to the inhibition of the gram-positive acid-forming organisms by the alkyldimethylbenzyl-ammonium chloride. The gram-negative rods are not inhibited and this accounts for the high bacterial counts. Qualitative evidence for this was obtained by identification of the organisms growing under the various conditions of test.

"Concentrations of surface-active cationic germicides varying from 1:500 to 1:20,000 can easily be determined in milk by titration with duponol PC in the presence of bromphenol blue." D.P.G.

MISCELLANEOUS

363. Rodent Control in Food Processing Plants. Donald H. Lewis, U. S. Fish and Wildlife Service, Ithaca, N. Y. 19th Ann. Report N. Y. State Assoc. Milk Sanit., p. 37. 1945.

Information is presented on the habits of rats and the damage which they cause. Modern rat control consists of four distinct methods, namely: (1) sanitation or elimination of food, (2) ratproofing buildings and elimination of exterior harborages, (3) killing by traps, poison, and fumigants, and (4) maintenance of the first three conditions. It was pointed out that red squill is the only rat poison that is not toxic to man and most domestic animals.

A.C.D.

364. Insect Control with DDT in Dairy and Milk Plants. Capt. R. S. Taggart, Sanitary Corps (R), U. S. Army, Washington, D. C. 19th Ann. Rept. N. Y. State Assoc. Milk Sanit., p. 21. 1945.

DDT was first synthesized in 1874 by a German chemist, Othman Zeidler. In 1939, Paul Muller in Switzerland was looking for an insecticide for the J. R. Geigy Company to control the Colorado beetle and he discovered the phenomenal powers of DDT. The U. S. Bureau of Entomology and Plant Quarantine, in 1942, tested numerous insecticides for war purposes and enthusiastically endorsed DDT.

The ordinary housefly and stable fly are killed by DDT within 30 minutes or more. The spray is not a repellent. The spray needs 3 to 5 per cent DDT and surfaces need good coverage. Tests show one spraying in early June and one in early August will keep barns free of flies. The residual effect lasts 1 or 2 months. The use of this spray does not reduce the need for other good sanitary measures. The spray is effective only against flies and not against eggs or larvae.

A 10% DDT mixture in talc will destroy cattle lice. This powder is effective against the American and German cockroaches but the results are not as spectacular or as complete as against flies. This powder is effective against fleas.

DDT may be safely handled with reasonable precautions. When fed in large quantities it kills animals and in regular small amounts it causes chronic poisonings. In oils it will be absorbed through the skin. It may be applied safely as a dust on animals. As a liquid to secure a residual effect, it should be sprayed in oil or as a water emulsion as a wet spray, but not as a fog. It may be applied with a brush. About 200 mg. of DDT are required per square foot of sprayed surface. A.C.D.

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CONTENTS

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Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
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Lancet
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Milk Plant Monthly

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

365. Advances in Enzymology. Volume 6. EDITED BY F. F. NORD. Published by Interscience Publishers, Inc., New York, 562 pages including cumulative index of Volumes 1–6. $6.50.

This sixth of an excellent series of reviews and discussions on enzymology prepared on invitation by authoritative workers includes sections on the following: Bacterial amino acid decarboxylases; Enzyme problems in relation to chemotherapy; Biological antagonisms between structurally related compounds; Adenosine triphosphatase properties of myosin, States of altered metabolism in diseases of muscle; Acetyl phosphate; Microbial assimilations; Chemical changes in harvested tobacco leaf; Actions of Amylases; Amylases of wheat and their relation to baking and milling technology; Tocopherol inter-relationships. The section on amylases of wheat contains an excellent review on the biochemistry of bread-making that will be of interest to those in dairy research. This and the previous five volumes on enzymology are specific tools for those in dairy research. K.G.W.

366. The Refrigerating Data Book. Refrigeration Applications Volume. Second Edition. THE AMERICAN SOCIETY OF REFRIGERATING ENGINEERS, New York, N. Y. 1946.

This volume has been prepared under the leadership of Donald K. Tressler, Chairman of the Board of Editors, made up of eight associate editors, each responsible for one of its eight parts. Eighty-nine specialists in the various branches of refrigeration applications contributed to the seventy-six chapters of the various parts. The eight parts are: I. Frozen Foods; II. Cold Storage Practice; III. Refrigeration in Food Manufacture; IV. Refrigerated Food Distribution; V. Low Temperature Applications; VI. Industrial Applications of Refrigeration; VII. Comfort Air Conditioning; VIII. Industrial Air Conditioning. At the head of each part is a list of chapter headings contained therein together with their authors. At the end of each chapter is found a reference list of detailed information sources. The 666 pages of subject matter contain many figures, graphs and tables. Chapters of paramount interest to the dairy industry are: 4. Ice Cream; 23. Milk Plants; 24. Butter Manufacture; 25. Cheese Manufacture, while others such as, 8. Bacteriology of Frozen Foods; 9. Nutritive Value of Frozen Foods; 10. Storage of Frozen Foods; 11. Cold Storage Plants; 12. Food Storage Conditions; 15. Egg Storage; 18. Ozone and Light; 20. Refrigerator Cars; 30. Refrigerated Storage of Dehydrated Foods; 32. Refrigerated Trucks; 33. Locker Plants; 34. Commerical Refrigerators; 35. Home and

Farm Freezers; 40. Dry Ice in Food Distribution; 41. Controlled Atmospheres; 46. Ice Making Plants; and 48. Dry Ice Manufacture may be of secondary interest. The publication contains a classified section of manufacturers of refrigeration equipment and supplies extending through 190 pages which should prove to be very useful as the publishers have endeavored to make the index accurate and authentic. L.M.D.

367. Introduction to Emulsions. GEORGE M. SUTHEIM. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1946.

A book upon the fundamentals of emulsion written by a practical man for practical men. The first five chapters are concerned with the fundamentals of emulsions and are: 1. Theoretical Foundation; 2. The Physical Chemistry of Emulsifying Agents; 3. The Chemistry of the Emulsifying Agents; 4. The Formation of Emulsions; 5. The Properties of Emulsions. Chapter 6 is a brief exposition of Applications of Emulsions. In addition to the frontispiece there are twenty-two figures, largely diagrammatic, illustrating various properties of emulsions. Each chapter is followed by a number of questions designed to bring out applications of its textual matter. Appendix I, Glossary. Terms peculiar to surface activity; Appendix 2, List of Emulsifying Agents. The information conveyed in this list consists of the commercial name, chemical name or formula, group classification, type, specific reference to bibliography, and producer's name in abbreviated form keyed to their full names in an accompanying list; Appendix 3. Bibliography. Contains a list of 159 titles extending from 1910 to 1945 but largely including references in the later years of 1935 through 1944. L.M.D.

368. Process Equipment Design. HERMAN C. HESSE, Professor of Engineering Drawing and Design, University of Virginia, AND J. HENRY RUSHTON, Professor of Chemical Engineers, University of Virginia. D. Van Nostrand Company, Inc., New York. 1945.

A book of particular interest to equipment and plant designers. Subjects covered as indicated by the eighteen chapters are: Materials of Construction; Mechanical Properties and Strength of Materials; Riveted Pressure Vessels; Welded Pressure Vessels; Mechanics; Threaded Fasteners and Combined Stresses; Structural Analysis; Trusses and Truss Adaptation; Piping; Attachments and Closures; Non-Ferrous Construction; Concrete Construction; Wood and Other Non-Metallic Construction; Belt and Chain Drives; Toothed Gearing; Shafting and Bearings; Handling Equipment and Mechanical Frames; Special Stress Application. References are grouped at the back of the book. Numerous illustrative figures, graphs, and tables and included. Wherever desirable sample calculations involving formulas and special applications are brought into the text to illustrate development of basic design. Each chapter has appended to it problems which in their solutions bring to bear information set forth in the chapter text. L.M.D.

369. Surface Active Agents. C. B. F. YOUNG, Director of Research, Clark Babbitt Industries, AND K. W. COONS, Head of Department of Chemical Engineering, University of Alabama. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1945.

The authors, recognizing that the success or failure of many industrial processes depends upon the effects of surface tension, have undertaken to present information as to the origin, effects, and utilization of surface-tension phenomena in a diversity of industrial fields. It is their hope, "that the transfer of knowledge from one field to another may bear fruit in easing the problems of some workers, or in providing the germ of an idea which may improve some process or solve some problem in another field."

A unique presentation in the first part of the book is a table of illustrations set up by chapters, carrying the figure number, title of illustration and page number.

Part I contains basic information embodied in three chapters. Chapter I, Theory of Surface Tension; Chapter II, Determination of Surface Tension; Chapter III, The Structure of Wetting Agents and Specific Surface-Tension Agents.

Part II consists of applications. Chapter IV, Emulsions; Chapter V, Plating, Metal Cleaning, Pickling, and Etching; Chapter VI, Cosmetics; Chapter VII, Leather; Chapter VIII, Flotation; Chapter IX, Inks; Chapter X, Textiles; Chapter XI, Cutting Oils; Chapter XII, Adhesives; Chapter XIII, Foods; Chapter XIV, Lubrication; Chapter XV, Soldering, Brazing, and Welding.

The latter part of Chapter III, extending from page 117 through page 152, consists of an alphabetical listing of Wetting Agents and Surface-Tension Agents, giving the name, type or chemical composition, use in industry, and the manufacturer. At the end of the table is a list of the manufacturers and their addresses.

Formulas for industrial application compounds are grouped at chapter endings preceding the references. L.M.D.

370. Physical Methods of Organic Chemistry. Volume I. EDITED BY ARNOLD WEISSBERGER, Research Laboratories, Eastman Kodak Company. Interscience Publishers, Inc., New York. 1945.

Present Volume I comprises sixteen chapters while Volume II, to follow, will contain ten additional chapters. The subjects are handled by twenty-nine contributor specialists in the various fields of physical methods applied to organic chemistry. The book is designed to furnish the specialist worker with information about physical methods of organic chemical problem solution with which he may not be at all familiar but that because of necessity in research work he must be forced to use. For the student it will serve to give him an insight into the many physical methods applied to organic chemistry. With the items listed in Volume II the complete coverage of physical aspects will be made. Chapter titles follow: I. Determination of Melting and Freezing Temperatures; II. Determination of Boiling and Condensation Temperatures; III. Determination of Density; IV. Determination of Solubility; V. Determination of Viscosity; VI. Determination of Surface and Interfacial Tension; VII. Determination of Properties of Monolayers and Duplex Films; VIII. Determination of Osmotic Pressure; IX. Determination of Diffusivity; X. Colorimetry; XI. Microscopy; XII. Determination of Crystal Form; XIII. Crystallochemical Analysis; XIV. X-Ray Diffraction; XV. Electron Diffraction; XVI. Refractometry. Volume II continues the chapter order with: XVII. Spectroscopy and Spectrophotometry; XVIII. Colorimetry, Photometric Analysis, and Fluorimetry; XIX. Polarimetry; XX. Determination of Dipole Moments; XXI Conductometry; XXII. Potentiometry; XXIII. Polarography; XXIV. Determination of Magnetic Susceptibility; XXV. Determination of Radioactivity; XXVI. Mass Spectrometry. Each chapter embracing a special subject has a detailed outline at its beginning, is liberally supplied with specific foot note references, and at its end a general reference list. In the 736 pages of the book there are in addition to numerous mathematical formulae 273 illustrative figures and 56 tables. L.M.D.

371. **Into the Freezer—and Out.** DONALD K. TRESSLER, Food Consultant; CLIFFORD F. EVERS, Research Director, Birds Eye-Snider Division, General Foods Corporation; LUCY LONG, General Electric Consumers Institute. Avi Publishing Co., Inc., New York. 1946.

Here at last is a book prepared by experts in the field of frozen foods for the layman, whom having had considerable experience with locker plant freezing and very limited operation of home freezers is about to become an important factor in the field of home freezer sales, once their production becomes free from restrictive shackles. Heretofore there has been a great amount of what could be called unorganized, and, even in part, unauthoritative information covering the various phases of selection, preparation and packaging, freezing and storage, and finally preparation for the table of vegetables, fruits, meats, poultry, and fish in the home. Recently there has been added to the list, partially prepared and wholly cooked bakery products, together with many cooked foods of other sorts. This book presents, in well-organized fashion and in readily comprehended language, the necessary information to insure success in the operation of a home freezer. The real answer to the home freezer operators' (both actual and contemplative) need for helpful and expert instruction for all kinds of foods freezing preservation. Chapter headings are indicative of what the reader will find: I. The Food of the Future Has a Past; II. Freezers are Revolutionary and Democratic; III. Your Locker Man and You; IV. A 4-Point Program for Freezer Space; V. The Hidden Merit of Frozen Foods: Better Nutrition; VI. "Big Five" for Fruits and Vegetables—Variety—Maturity—Speed—Packaging—Storage Temperatures: How These Affect Success of Freezing—Planting and Harvesting Guide; VII. Step-By-Step Preparation Procedure; VIII. A Delicatessen at Your Fingertips—Cooked Foods—Baked Goods—Leftovers; IX. When Foods Come Out of the Freezer—How to Thaw, Cook, Use; X. Ice Cream in the Freezer Too!—How to Make and Package Ice Cream for the Freezer—How to Make "Ribbon" and Fruit Ice Cream—Velva Fruit; XI. Freezing Wild Game, Meat, and Fish. A number of photographic illustrations of procedure are used to supplement the instructional text. L.M.D.

BACTERIOLOGY

372. The Activity of Penicillin in Relation to Bacterial Spores and the Preservation of Milk.

HAROLD R. CURRAN AND FRED R. EVANS, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr., Washington, D. C. Jour. Bact., *52*, No. 1: 89. July, 1946.

This is a study of the preserving action of penicillin in milk containing viable bacterial spores. Fifteen aerobic and two anaerobic species of bacteria were examined.

Four species of the genus *Bacillus* were relatively resistant; penicillin was not an effective preserving agent except in an impracticable concentration. Spores of the remaining 13 species were either killed or inhibited by penicillin concentrations of 5 units per ml. This concentration sterilized many cultures. Five units delayed but did not prevent spoilage by *Clostridium botulinum* and another unidentified anaerobic species. Apparently all spore cultures contain some spores susceptible to penicillin; the species differ in the proportion of resistant and sensitive cells.

It was concluded that penicillin has no application in the preservation of food. Accompanied by mild heating it might serve as a preservative in certain nonfood materials. D.P.G.

373. The Activity of Streptomycin in Relation to Bacterial Spores and the Preservation of Milk.

HAROLD R. CURRAN AND FRED R. EVANS, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr., Washington, D. C. Jour. Bact., *52,* No. 1: 142. July, 1946.

This is a note dealing with a study similar to that reported on penicillin by the same authors. Many spore-forming bacteria grew in the presence of

5 units of streptomycin per ml. of milk. One hundred units per ml. were not sufficient to prevent spoilage of milk containing as few as 100 per ml. viable spores of *Clostridium botulinum* and of another anaerobe. It is concluded that streptomycin in ordinary concentrations has limited activity against bacterial spores. D.P.G.

374. The Action of *Leuconostoc dextranicum* and *Leuconostoc citrovorum* During the Ripening Process of American Cheddar Cheese. CHARLES C. PROUTY, Department of Bacteriology and Public Health, State College of Washington, Pullman, Wash. Jour. Bact., *52*, No. 1: 153. July, 1946. Proc. of Local Branches.

D.P.G.

CHEESE

375. The Centrifugal Milk Clarifier in Cheddar Cheese Manufacture. E. G. HOOD AND I. HLYNKA. Canadian Dairy and Ice Cream Journal, *24*, 5: 27. May, 1945.

The three main points brought out in favor of clarification of cheese milk are: (1) removal of sediment; (2) improvement in flavor and texture; (3) reduction of fat loss in the whey. H.P.

376. Keeping Quality of Cheese With Rancid and Unclean Flavours. I. HLYNKA AND E. G. HOOD. Canadian Dairy and Ice Cream Journal, *23*, 11: 35. November, 1944.

A comparison of data on the flavor scores of three different lots of cheddar cheese at 2, 4, 6 weeks and at 6 months show that cheese made from unagitated milk maintained its flavor score throughout the ripening period. Cheese made from milk which was subjected to agitation so as to provide varying intensities of rancid and unclean flavors on the whole maintained its flavor scores up to 6 months though the results were variable. It is concluded that milk lipase is not active in cheese after it is made, and variations in flavor score are attributed to other factors. H.P.

377. Testing Cheese for Extraneous Matter. ARTHUR B. EREKSON, Plymouth, Wisconsin. Natl. Butter and Cheese Jour., *37*, No. 8: 46. August, 1946.

A special device with a V-shaped blade is used to cut an 8-ounce piece of rindless cheese from a flat surface of a Cheddar. The cut surface of the cheese is sealed by the usual operation of paraffining. Damage by mold growth during curing is eliminated by this system. The sample is shredded rather than ground; it is dissolved in a 10% sodium citrate solution at 155° F.; finally, it is filtered in an Erekson vacuum sediment tester. Sediment discs are dried, placed in cellophane envelopes and examined for amount and types of extraneous material. Discs are graded according to standards adopted by the National Cheese Institute. Routine testing is the only basis for control of extraneous matter. W.V.P.

CHEMISTRY

378. pH and Its Many Applications in the Dairy Industry. L. R. BRYANT. Canadian Dairy and Ice Cream Journal, *24*, 4: 27. April, 1945.

There are many applications of pH in the dairy industry. The most common pH measurements are used in: (1) milk (normal pH values between 6.5–6.7); (2) cream and butter; (3) cheesemaking; (4) casein manufacture; (5) refrigeration brines; (6) boiler waters and (7) washing solutions. As the influence of pH on many dairy problems becomes more generally recognized and understood by dairy plant operators, the measurement of pH will be more widely used in dairy plant practice. H.P.

379. Use of an Ion-Exchange Resin in Determination of Traces of Copper With Special Reference to Powdered and Fluid Milk. H. A. CRANSTON, Lab. of Polarographic Analysis, Chicago, Ill., and J. B. THOMPSON, Quartermaster Food and Container Institute for the Armed Forces, Chicago 9, Ill. Jour. Ind. Eng. Chem., Analyt. Ed., *18*, No. 5: 323–326. May, 1946.

The copper content of a good dry whole milk is generally less than 1 part per million and this small concentration of metal makes the determination of copper difficult. The official army specification method of analysis lacks precision particularly at the critical range below 2.0 p.p.m. In current methods of analysis all the organic matter must be destroyed in large samples by acid digestion or ignition. This is avoided in the new method, the copper being made available by reducing the reaction to less than pH 3.0 with perchloric acid. The precipitated milk protein, carrying the fat with it, is removed by filtration. The filtrate is corrected for added acid, solids, and fat loss. The copper from this filtrate is concentrated on a synthetic ion-exchange resin in the hydrogen cycle. Copper is stripped from the ion exchanger with dilute hydrochloric acid and determined polarographically using an additive standard technique. If polarographic equipment is not available, spectrophotometric methods may be employed. Data showing the precision of the proposed method and a comparison of it with other methods for determining copper are given. B.H.W.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

380. Freezing and Storing Condensed and Skim Milk. D. V. JOSEPHSON, Department of Dairy Technology, Ohio State University. Ice Cream Field, *48*, No. 1: 37. July, 1946.

Results of experimental work are reported showing that condensed whole milk and condensed skim milk can be frozen and stored for several months without seriously affecting their quality.

The following procedure is recommended:

Select high quality whole milk and forewarm it to 190° F. for 15 minutes, then concentrate it to 39 per cent total solids (3 to 1 ratio). Cool to 40° or less and run into tinned or paper cans. Freeze as rapidly as possible and store at 10° F. or lower.

Several test cartons (quart or gallon size) should be frozen and stored under the same conditions. At intervals from three months on remove one of these cartons and test its dispersability in hot water (180° F.) in a malted milk mixer. If curd resists dispersion, the lot should be removed and used immediately.

Skim milk should be concentrated to 36 per cent total solids (4 to 1 ratio) otherwise it is processed essentially as the condensed whole milk.

The "cooked" flavor present in these products when first processed serves to prevent "oxidized" flavor from developing. It also tends to disappear during storage. W.C.C.

DISEASE

381. Control of Brucellosis in New York State Dairy Herds and Its Relation to Milk Supply. ASA WINTER, N. Y. State Dept. Agr. and Mkts., Albany, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 79. 1945.

Of all herds in New York State under the test and slaughter plan that were approved as being free from the disease on January 1, 1944, more than one-half revealed infection during the 15-month period ending April 1, 1945. These outbreaks were probably greater than normal.

In research reported by the New York State Veterinary College vaccinated calves were exposed to infection and field strains of *Brucella abortus* (not strain 19) were isolated from the milk of some heifers that remained as persistent reactors.

The program of controlling *Brucella* in New York State herds follows two plans. Plan A provides for vaccination of calves and annual blood tests of the herd. Plan B provides for heavily infected herds by vaccination of calves and also adult vaccination to reduce abortions. When a herd under Plan B becomes sufficiently negative it may be blood tested, reactors removed, and transferred to Plan A. A.C.D.

382. The Diagnosis of Chronic Brucellosis. Current Comment, Jour. Amer. Med. Assoc., *131*, No. 8: 670. June 22, 1946.

This is a thumbnail summary of present day knowledge of chronic brucellosis. Brucellosis should receive consideration early in the diagnosis of obscure illness. There is not yet available any easy adequate specific means of diagnosis of chronic brucellosis. The agglutination test is significant only if positive. A positive intradermal test indicates increased sensitivity, but does not reveal whether the infection is still active. Negative skin tests have been observed in active brucellosis infection. The opsonophagocytic test requires perfect technic with a virulent brucellosis strain. Increased phagocytosis with a positive skin test, and symptoms suggesting brucellosis, point to a still active infection. The isolation of brucellosis by culture from the patient is the only definite diagnostic procedure. Cultural methods now in use are hardly practicable for daily routine work in the clinical laboratory. A high incidence of arthritis in brucellosis is believed to exist. In one study about half of 157 arthritis patients were believed to have brucellosis. Another observer has reported that of 427 patients with brucellosis, 74 had arthritis. Systematic study of the relation of brucellosis to chronic arthritis should be promoted. Among localizations of chronic brucellosis are those in the female genitourinary tract. More adequate cultural study with special reference to the detection of brucellosis is essential to a better understanding of various chronic infections. Cooperative investigation of the problems of diagnosis and treatment are needed. D.P.G.

383. The Incidence of Staphylococcal Mastitis in the Northwest.

ERNEST C. MCCULLOCH, State College of Washington, Pullman, Wash. Jour. Bact., 52, No. 1: 153. July, 1946. Abs. Proc. of Local Branches.

"A survey of over 3,000 cows in Washington revealed 34 per cent to have one or more quarters showing some degree of abnormality. Of the incubated milk smears examined and quarter samples cultured, 64 per cent contained staphylococci and 28 per cent streptococci, the remainder being contaminants or miscellaneous types of infection.

"The pathogenicity of staphylococci was checked by streaking on 5 per cent bovine blood agar, and after 24 hours' incubation at 37° C. by streaking the hemolytic colonies in Difco phenol red mannitol agar to which was added an additional 70 grams of NaCl per liter. Staphylococci capable of hemolyzing bovine blood, growing on 7.5 per cent salt agar, and utilizing mannitol have been considered as pathogenic.

"The infusion, immediately following milking, of 25,000 to 50,000 units of penicillin into the teat canal of quarters shedding staphylococci was followed by a temporary inability to culture staphylococci from the milk, but even the infusion of 100,000 units, repeated four times after four successive milkings, failed in several quarters to prevent the reappearance, after 7 to 14 days, of staphylococci in the milk.

"The plate counts of penicillin-treated quarters show a tendency to approach gradually the levels of staphylococci found previous to treatment."

D.P.G.

384. ***Salmonella typhimurium*** **Food Infection From Colby Cheese.** C. B. TUCKER, M.D., GEORGE M. CAMERON, PH.D., MATTIE P. HENDERSON, M.S., AND M. R. BEYER, M.D., Tennessee Department of Public Health, Nashville, Tenn. Jour. Amer. Med. Assoc., *131,* No. 14: 1119. August, 1946.

Two hundred and fifty human cases of food-borne infection occurred in an epidemic involving six towns. History of the food eaten revealed that all patients had eaten Colby cheese 24 to 48 hours prior to the onset of the illness. Family members who did not eat cheese remained well. *Salmonella typhimurium* was isolated easily. Subsequent investigation revealed that a mouse had been removed from a ten thousand pound vat of milk which was made into cheese.

The cheese moved in interstate commerce and thus attracted the attention of the U. S. Food and Drug Administration. Six pounds of the cheese was acquired from a grocery store for bacteriologic study. It had been kept at 50° F. by the groceryman. In the laboratory the cheese was held at 43 to 48° F. Bacteriological isolations were made at monthly intervals. A small surface area was removed with a sterile scapel. A portion of cheese taken aseptically from beneath this area was macerated in a bottle containing selenite F liquid media and incubated at 37° C. for 24 hours. Several loopfuls of selenite F broth culture were then streaked on S. S. agar plates. After 24 hours at 37° C. typical colonies were transferred to Krumwiede's triple sugar agar slants and incubated 24 hours at 37° C. Further identification was obtained by incubation of the appropriate differential culture media. Final verification was secured by the assistance of the Salmonella Center, University of Kentucky. *S. typhimurium* remained viable in this cheese for a period of 302 days at 43 to 48° F.

It is suggested that pasteurization of milk and cheese curd in the process of cheese manufacture should be carried out. It is believed that other sanitary measures should be strictly enforced in the manufacture of cheese. Pasteurization of the milk alone would not have prevented the occurrence of this outbreak. D.P.G.

FOOD VALUE OF DAIRY PRODUCTS

385. Vitamin C in Milk Products. Current Comment, Jour. Amer. Med. Assoc., *131,* No. 10: 828. July 6, 1946.

A considerable part of the daily requirement of ascorbic acid is contributed by fresh milk. A quart of milk may contain 22 mg. of reduced vitamin C, almost a third of the recommended allowance. Commercial handling and processing drastically reduces this value. One study indicates that pasteurized milk from consumers' homes and retail stores averaged 5.8 mg. per liter, while reconstituted evaporated milk averaged 2 mg. Fresh powdered whole milk after being reconstituted averaged 12.5 mg. per liter, and the vitamin C in the dried milk was retained after 12 months at room temperature to the extent of 80%. D.P.G.

ICE CREAM

386. A Roadside Ice Cream Business. Ice Cream Field, *48*, No. 1: 18. July, 1946.

The success of roadside stands depends upon many things. Mention is made of the importance of location, the advisability of adequate parking to encourage profitable curb service as well as proper layouts for attractive and efficient stands.

The balance is from an article by Charles W. Alexander which appeared in *Printers Ink.* Alexander states that millions of people travel the "open road" and this will affect many phases of manufacturing, mediums of advertising as well as sales methods. He warns against doing the wrong thing when starting a roadside business and stresses the following:

1. Pick spot carefully. Traffic count alone is not enough, ratio of customer cars to total traffic is important. It is stated that "Roadside business is much more a creature of the weather than metropolitan outlets."
2. It is vitally important to know the type of road. It is claimed that traffic lights are an asset and a corner location of important intersecting highways is desirable.
3. The road may change, hence it is important to contact the State Highway authorities, especially the road inspector under whose jurisdiction your prospective site will come. He can be of considerable help.
4. Pick the outside of a curve but not a winding section of the road likely to be replaced by a straight one.
5. The apex of a forked road is a good location.
6. Check zoning restrictions.
7. Check land characteristics and drainage as well as availability of electrictiy, gas and telephone.
8. Suitable sized plot with ample frontage as well as good landscaping are important.
9. Building should be designed by experienced architect and be air-conditioned.
10. Operating costs should be considered in deciding whether to lease or buy. All agreements should be in writing.
11. Radio and mail advertising can be effective in promoting sales. Names of car owners who frequent your stand can be secured from the State Registrar of Automobiles. W.C.C.

387. Nectar Research Progressing. W. V. CRUESS AND I. G. A. GLAZEWSKI, Division of Food Technology, University of California, Berkeley, California. Ice Cream Field, *48*, No. 1: 42. July, 1946.

Results of experiments on fruit purees and nectars are briefly described. Apricot nectar, consisting of 50 per cent apricot puree and 50 per cent light sugar sirup, was originated about fifteen years ago. It has been produced commercially in sterilized form, but the frozen nectar is also an excellent product with many uses, the authors claim.

Several blends were found to be very pleasing. Thus apricot nectar 2 parts, apple juice 1 part and 6.6 per cent lemon juice was very palatable. Likewise a blend of Valencia orange juice and apricot juice was pleasing. Pineapple juice blended with apricot nectar required acidification for best results. Blends of pineapple juice and apricot puree were too viscous.

Good peach nectars were also made from the following varieties: J. H. Hale, Elberta and Rio Oso Gem. Acidification to 0.3 per cent to 0.4 per cent citric acid was beneficial.

Pear-peach nectar blends, plum nectar and several other blends were found pleasing in flavor. W.C.C.

388. Preparation and Pretreatment of Fruits for Freezing. LEONORA A. HOHL, Division of Food Technology, University of California, Berkeley, California. Food Freezing, *1*, No. 8: 287. June, 1946.

A summarization of research to date on the control of changes in color, flavor, and texture in fresh frozen fruits. Much progress has been made in the direction of retaining natural color and flavor but it appears that knowledge of the complex physico-chemical or colloidal changes which take place during the processes of freezing and thawing is insufficient to make specific recommendations concerning the most effective way of preserving the natural texture of fruits. In general, it is probably true that the importance of this reason for quick freezing has been overemphasized. Minimization of chemical and microbiological changes are still good arguments in favor of rapid freezing. Pretreatments of fruits for freezing to minimize or control oxidation fall into four general categories: (1) selection of suitable varieties, (2) exclusion of air, (3) anti-oxidants, (4) blanching. Two tables are included, I. Recommended Quantities of Ascorbic Acid for Inhibition of Browning in Frozen Fruits, II.Condensed Directions for Preparation of Important California Fruits for Freezing. L.M.D.

MILK

389. Effect of Cooling on Extent of Fat Dispersion in Agitated Milk. E. G. HOOD. Canadian Dairy and Ice Cream Journal, *21*, 4: 35. April, 1945.

Morning's milk was cooled at 85°, 75°, 65°, 55°, and 45° F. Sixteen gallon lots of milk taken at each temperature were agitated by churning for some 5 minutes, warmed to 85° F., and separated. A sample of each lot of skim milk was analyzed for butterfat. In a second set of experiments evening's milk which had been cooled to below 45° F. was warmed up to each of the above temperatures and the same procedure followed. As the temperature at which milk was agitated became progressively lower, the butterfat content of the skim was also lower. On the basis of previous work the results are interpreted to mean that vigorous agitation of milk causes larger fat globules to break up but that this effect is minimized by cooling the milk to below 55° F. H.P.

390. Transfer of Unsatisfactory Dairies Between Health Jurisdictions. CARSON H. OUTWATER, Dept. of Health, New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 159. 1945.

As no recognized health agency desires unsatisfactory dairies under their jurisdiction it is suggested that dairies desiring transfer should be carefully inspected. It is necessary to (1) keep control records, (2) require application for transfer with pertinent prior record, (3) require investigation and inspection before permitting transfer, and (4) in questionable cases require letter of authorization from health agency which the dairyman wishes to leave. A.C.D.

391. Postwar Milk Bottle. V. L HALL. Glass Container Mfg. Inst., New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 155. 1945.

The square milk bottle is being strongly recommended to the dairy trade in all sizes. The quart size weighs 17¾ ounces and has an opening of 51 mm. or less. Satisfactory washing and breakage are no problems as compared with the round bottle. A case of round bottles occupies 47½% more space than a case of square bottles. In a refrigerator 12 square bottles occupy the space of 8 round bottles. A.C.D.

392. The Future of Fiber Milk Containers. FRED C. BASLET, American Can Co., New York, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 145. 1945.

In 1944 there were 375 dairies in the U.S.A. using 2,000,000,000 fiber milk containers. They were used chiefly in stores. The percentages of all store accounts sold in paper were Chicago 80, Los Angeles 75, San Francisco 60, and New York 45. About 60% of all housewives prefer paper. The trend toward homogenized milk has tended to minimize the objection to paper containers that the cream layer does not show.

Three types of paper containers are used, namely, (1) those formed and paraffined in the dairy as needed, (2) those which are pre-fabricated and require special machines for filling, and (3) those which are pre-fabricated and may be filled in ordinary glass milk bottle machines.

Cost is vital. At one cent each, an increase of one mill per container means an increased cost to consumers of $3,000,000 per year. If costs double, few paper containers will be used, but if costs could be cut in half then nearly all milk would be sold in paper. A.C.D.

393. Problems in Design, Installation, and Operation of H.T.S.T. Pasteurizers. C. W. WEBER, N. Y. State Dept. of Health, Albany, N. Y. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 121. 1945.

There is need to standardize the design and installation of H.T.S.T. pasteurizers to assure most perfect results. From a public health standpoint the H.T.S.T. pasteurizer equals or exceeds any other type of pasteurizer. Attention needs to be given to certain phases of the process as (1) heating every particle of milk to 160° F. or higher, (2) holding every particle for 15 seconds or longer, (3) prevention of contamination of properly pasteurized milk with pre-pasteurized milk, (4) installing homogenizer in the system, (5) cleaning and sterilizing, and (6) handling breakdowns during operation. A.C.D.

394. Preliminary Report on Coliform Studies of Pasteurized Milk and Milk Products. LEON BUCHBINDER AND JOHN W. FERTIZ, N. Y. City Dept. of Health, and School of Public Health, Columbia University, New York City. 19th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 103. 1945.

This study was conducted on some 2,250 samples of pasteurized milk collected at the bottle fillers or in the refrigerators of 46 plants in New York City.

It was found that the coliform counts were much higher in the summer than in the winter. There were 400 quarts of milk collected at the pasteurizers and only one sample was coliform positive, thus proving that coliform bacteria in pasteurized milk represent recontamination. Very little difference was obtained by using sodium desoxycholate agar and brilliant green bile broth.

All milk samples were plated in 1-ml. quantities, also 20 ml. milk were divided in 4-ml. portions and poured into 5 plates, and finally the remaining quart of milk (900 ml.) was incubated overnight at 37° C. and then plated in 1-ml. quantities. Tests showed that 2 to 5 coliform bacteria per quart of milk would give positive for coliform after incubation overnight at 37° C. The data showed that most milk was coliform positive if the samples were large enough, and the number of coliform bacteria increased with an increase in the mean outdoor temperature.

Analysis of the data lead the authors to think that two standards for coliform counts should be used, one for May through November, and a lower count for November through April. Trials are being made to try the application of 10 or more colonies per 5 ml. of milk in summer and 6 or more colonies per 20 ml. of milk in winter as representing unsatisfactory milk.

A.C.D.

MISCELLANEOUS

395. Tomorrow's Packages. An open letter to materials manufacturers.

C. I. Sayles, Associate Professor, Institution Engineering, School of Nutrition, Cornell University. Food Freezing, *1*, No. 7: 246. May, 1946.

A plea is made for standardization of the moisture-vapor test for wrapping materials used in frozen foods packaging in order that the customer whether locker plant operator or processor will be in a position to exercise selection of protective wrappers on a comparative basis. The recommendation made contains four points for test standardization: (1) Moisture-vapor results be reported in the same units of weight and area; (2) That a common relative humidity test condition be chosen as a standard; (3) That a standard 0° F. test be sought; (4) That eventually all these results shall be brought to the public in a simple form related to the length of storage time so that a particular wrapping material may be designated as a "six months" or a "year paper."

L.M.D.

396. Sanitation in the Processing Plant and Its Relation to the Microbial Quality of the Finished Product.

Reese H. Vaughn and Thressa C. Stadtman, Division of Food Technology, University of California. Food Freezing, *1*, No. 7: 334. July, 1946.

Proper blanching of vegetables will reduce the number of microorganisms at least 99.9 per cent. Post-blancher contamination is the vital factor that the processor must keep in mind. Contaminated cooking water, conveying belts, and miscellaneous handling equipment should be guarded against. Even the human element should be considered. Continuous sanitizing of equipment such as belt conveyors and elevators is recommended. Sanitizing agents that may be used are: (1) chlorine in residual strength above breakpoint to be great enough to destroy microbial growth and prevent slime formation; (2) sulfiting by means of SO_2 gas or sulfite salts; (3) surface-active agents possessing disinfecting powers as well as detergency. Diagrams are included showing methods for applying sanitizing solutions to conveyor belts.

L.M.D.

397. Handling of Long-Distance Shipments of Frozen Foods. Edwin Smith, Senior Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, ARA, U.S.D.A. Food Freezing, *1*, No. 7: 331. July, 1946.

A discussion of results observed in long-distance shipment of frozen foods in various types of refrigerator cars. Many details are touched upon which indicate that the refrigeration protection of frozen foods in transit present new problems compared to the older standard practices in vogue for many years for handling frozen meat carcasses and cold pack fruits. In general the best results in temperature control were obtained with the "Preco" fan car and the overhead brine tank car. Emphasis is given to the need for improvement in handling at loading and receiving ends to keep down the temperature rise of car on the one hand and the temperature rise of the goods on the other. One important fact was brought out that goods thought to be "zero or lower" often were in the twenties. This condition puts undue load upon the car's refrigeration facilities. Car design must be such that packages are not stowed in direct contact with car walls. Heavy insulation is a must for long-distance transport because cars spend a large percentage of the overall haulage time actually standing idle, which in the case of the fan car means air circulation ceases. In this case the overhead brine tank car offers an advantage in that the refrigeration effect is obtained through convection. L.M.D.

Abstractor's comment: Forced convection with the overhead brine tank would seem to offer some advantage where close storage of cartoned goods is practiced.

398. Studies of Frozen Food Samples Bought in Open Market. Part I. Observations on color, texture, appearance and odor of frozen vegetables. W. V. Cruess, Professor of Food Technology, University of California. Food Freezing, *1*, No. 7: 243. May, 1946.

The quality of frozen vegetables that the consumer is receiving is an important factor in their merchandising, a phase of the retailing of frozen foods which should be given increased attention. The author used in his investigations a score card containing the items, Color and Appearance—20, Texture—20, Flavor—45, and Odor—15, Total 100. Under the heading "Comments," are included a description of the odor and flavor, state of maturity of the vegetable, character of color, and the degree of catalase and peroxidase activity. Also a check of actual weight found against declared net weight. The procedure was first to thaw at room temperature and then test for catalase and peroxidase. The vegetables were then cooked either in steam or in a pot with a very small amount of water; then were tasted.

Ten tables of score results on different vegetables are included together with comments. In general the results indicate considerable chance for improvement in quality under all items of scoring and a need for control of stage of maturity in selection of vegetables for freezing. L.M.D.

399. The Rôle of Water in Freezing Foods. MILO R. DAUGHTERS AND DAWRENCE S. GLENN, Western Research Laboratories, Frosted Foods, Inc. Refrigerating Engineering, *52*, No. 2: 137. August, 1946.

As a result of the beneficial results observed when figs that have begun to shrivel are frozen (they withstand freezing better and have better texture when defrosted than do those that are in a plump and succulent stage of maturity) the authors have advanced the theory that bound water is the responsible factor. The assumption that all moisture in foods is changed to ice crystals at sub-zero temperatures may not be correct. According to the theory only free water is changed to ice and the bound water is held in some form which either does not freeze at the temperatures employed in quick freezing, or, if it does, freezes to crystals of molecular size which do not rupture the tissue. Once the methods of reducing the percentage of free water in foods, or the means for transforming it into bound water are known, there should be a decided improvement in the quality of many of the frozen foods now coming into the market.

The authors, employing the calorimetric method, determined the proportional distribution of free and bound water in several fruits and vegetables. It is a possibility that partial dehydration may become a processing step in commercial food freezing which could be combined with deactivation of enzymes. L.M.D.

400. Evacuation and Dehydration of Field Installations. HOWARD A. BLAIR AND JOEL N. CALHOUN, B. F. Sturtevant Company, Division of Westinghouse Electric Corporation, Boston, Mass. Refrigerating Engineering, *52*, No. 2: 125. August, 1946.

The authors review the means for removal of moisture from Freon refrigeration systems, the undersirable effects of excessive moisture and recommend a combination of vacuum evacuation and dehydration of installations in the field as the most efficient means of moisture removel where Freon-12 is concerned. L.M.D.

401. Refrigerated Transport. PAUL B. REED, Perfex Corporation, Milwaukee, Wis. Refrigerating Engineering, *52*, No. 2: 115. August, 1946.

A comprehensive survey of the field covering rail, motor, and air refrigerated transport. Most recent developments in attempts to develop efficient and economical refrigeration applications in the transportation of perishable foodstuffs are described. The author believes that the present state of refrigeration equipment of cars, trucks, and trailers, much of which is antiquated and badly worn, offers great opportunities for exploitation, development, and expansion. L.M.D.

402. The Control of Microorganisms in Food Storage Rooms. W. L. MALLMAN AND E. S. CHURCHILL, Section of Bacteriology and Public Health, Michigan Agricultural Experiment Station, East Lansing, Mich. Refrigerating Engineering, *51,* No. 6: 523. June, 1946.

Ultraviolet rays were found to produce reduction of bacteria on surfaces directly irradiated, followed by a gradual increase after prolonged irradiation. In closed rooms, surfaces shielded from direct irradiation showed some reduction in surface populations. In closed rooms ozone atmospheres gave results comparable to those obtained with shielded ultraviolet rays.

Carbon dioxide in laboratory tests in concentrations of 2.5 per cent suppressed the rate of growth of bacteria on surfaces, while those of 5 and 10 per cent caused reductions in populations of 45–90 per cent depending upon the type of bacteria.

Glycols when vaporized into the air caused reductions of 40–90 per cent, but had no lethal action upon spores.

Ultraviolet rays, ozone, carbon dioxide and glycols may be classified as marginal sanitizing agents which have specialized use in refrigerated and food handling rooms. In all instances they should be tested out fully for each specific application before permanent installations are made.

Both hypochlorites and quaternary ammonium chlorides are applicable to surface disinfection in food preparation and storage rooms, but due to the fact that surface sanitizers have only a limited action on bacterial spores, gross contamination of spores must be removed by thorough washing and flushing of surfaces in order to achieve successful results in maximum reduction of surface contamination. L.M.D.

403. Technical Phases of Home Freezer Development. C. E. LUND, Director of Research, Seeger Sunbeam Corp., St. Paul, Minn. Refrigerating Engineering, *51,* No. 6: 513. June, 1946.

Emphasis is placed upon the design of freezer cabinets from the standpoint of economics of operation. It is brought out that four inches, five at the most, is the limit in insulation thickness. Beyond this, additional thicknesses result in no economy of operation or in prevention of surface condensation ("sweating"), nor was insulation beyond four inches of thickness warranted when considering maximum storage space against external dimensions. Top thickness and that of lids can be set at about two and one-half inches. Vitally important is vapor sealing. Whether double gaskets or single gaskets are used on lids there must not be any appreciable vapor leakage. Care should be exercised to use materials for obtaining structural strength and rigidity between the inner and outer walls of a freezer cabinet. A table (3) of performance characteristics for four capacities of home freezer cabinets operating at 0° F. cabinet temperature is included. The cabinets are of 6, 9, 12, and 18 ft. sizes. At two and one-half cents per kwh at 70° F. the costs were per month, 0.84, 1.27, 1.50, 1.76 dollars, respectively, while at 100° F. they stepped up to 1.86, 2.24, 2.65, 3.10 dollars. L.M.D.

404. Tomorrow's Packages—and Their Merchandizing Value in Display. J. Alfred Anglada, Sylvania Industrial Corp. Food Freezing, *1*, No. 6: 220. April, 1946.

Stress is placed upon the fact that a frozen food package must possess sales appeal in addition to affording protection against deterioration of its contents. In providing an attractive package, cellophane not only allows visual appraisal of contents through the media of window sections, lift tops, window tabs, etc., but, if properly sealed, insures the food product against dehydration, oxidation and under some conditions added moisture. The multiple unit package is advocated as a merchandising unit for complete frozen meals, or a number of units of the same food. Cellophane as an overwrap lends itself to printing, particularly on the reverse side which enhances the beauty of the print job by lending gloss and depth of color to the ink. Sealing cellophane by heat calls for one second or less at 250° F. to 300° F.
L.M.D.

405. Studies of Frozen Food Samples Bought in Open Market. Part II. Observations on the color, texture, appearance and odor of frozen fruits. W. V. Cruess, Professor of Food Technology, University of California. Food Freezing, *1*, No. 8: 306. June, 1946.

Part I, on frozen vegetables, appeared in Food Freezing, May, 1946. Samples of frozen fruit purchased in the open market were subjected to scoring upon a rating basis of—color and appearance 20, texture 20, flavor 45, and odor 15 for perfect score. Three tables showing results for apricots, apple sauce, and sliced peaches are included. Orange juice and other fruits are commented upon. In general it is indicated that much improvement remains to be made if frozen products are to compete with fresh and canned one. The author recommends flash pasteurization of citrus juices following vacuumizing before packing and freezing. Fruits should be covered with syrup. In the case of vegetables more thorough blanching should be practiced. Fluming may be responsible for a large part of the loss of flavor of vegetables and apples. Net weights of fruits were generally over declaration because of added syrup but practically all vegetables were below declared net weight, shortages of 1 oz. being common. This indicates a need for protective packaging that is more efficient in preventing moisture vapor passage. The loss is probably through sublimation of ice. L.M.D.

406. Illinois Co-op Locker Associations "Come of Age." THEODORE H. KIMBLE. Food Freezing, *1*, No. 8: 292, 314. June, 1946.

An exposition of the results of a field survey of 25 cooperative locker plant associations in 25 Illinois counties made by Paul C. Wilkins of the research division of the U. S. Dept. of Agriculture's Farm Credit Administration in cooperation with the St. Louis Bank of Cooperatives. The 25 associations constituted 48 complete plants and 12 branch rooms. The complete plants had an average capacity of 510 lockers; the 12 branch rooms (without processing equipment) averaged 203 apiece. A total of 26,975 lockers were being rented at the close of the fiscal year last July 31. Tables, Comparative Analysis of Expenses Per Locker Rented and of Income Per Locker Rented are given showing the high, low and average figures itemized. The average total expense was $21.03, average total income $23.09, resulting in a net saving of $2.06. It is evident from the average income from product items that the bulk of services is in connection with meat products, relatively small returns being received from vegetable and fruit processing and poultry. Two major difficulties encountered by most associations in operating their processing rooms at a profit were: (1) Insufficient volume of products in one or more of the plants. (2) Wide fluctuations in volume from month to month. Many associations are considering consolidation of processing plants in one centralized location and converting processing and high temperature rooms to additional low temperature storage space. Also the increase of poultry processing in the summer months together with freezing fruits and vegetables for re-sale out of season to locker patrons and other local sales outlets should offer a means of fuller utilization of centralized processing plant personnel and equipment. L.M.D.

407. Tomorrow's Packages—and the Use of Petroleum Waxes. J. F. BUTTERWORTH, Staff Engineer, Process Products, Socony Vacuum Oil Co., Inc. Food Freezing, *1*, No. 8: 296. June, 1946.

The most universally used packaging material—be it in frozen food or in other food requirements—is *paper treated with petroleum wax.* There are two broad classifications of waxes, namely, paraffin-type waxes and microcrystalline type waxes. The former are hard, brittle, white, and crystalline; while the latter are generally darker in color, have great ductility at all temperatures, are tacky, have smaller crystals and higher melting points. For low temperature use in frozen food packaging refined paraffin waxes are blended with microcrystalline waxes to obtain low temperature flexibility. Converting paper with waxes can be grouped into three classifications: 1. "Dry-Waxed" or impregnated papers; 2. "Wet-Waxed" or surface-coated papers; 3. Laminated papers.

While dry-waxed papers are waterproof for practical purposes they are not moisture-vapor barriers. Wet-waxed papers possess moisture-vapor-proofness and if the paraffin wax has a small quantity of microcrystalline wax blended with it the coating will have the necessary ductility for low temperature use. Laminated papers making use of microcrystalline wax combine the desirable characteristics of two materials. L.M.D.

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CONTENTS

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Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

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Milk Industry
Milk Plant Monthly

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ABSTRACTS OF LITERATURE

BUTTER

408. Essentials to the Improvement of the Quality of Cream. F. W. HAMILTON. Canad. Dairy and Ice Cream Jour. *25*, 5: 33. July, 1946.

The problem is the production of better raw cream. This can be accomplished by (1) increase in production of butterfat per acre, (2) healthy cows properly fed and cared for in clean surroundings, (3) proper cooling of the cream and (4) handling in sterilized utensils. Greater volume and more profitable production means better quality. Standards for sanitary production must be established. For marketing of cream to creameries a cream producer should meet the following requirements: (1) he must be fully aware that he is producing a food for human consumption, (2) only healthy cows be maintained in the milking herd, (3) stables with cement flooring and adequate drainage and lighting be provided, (4) a separate building or room be provided for separating of milk and storage of utensils, (5) that adequate means of cooling cream be provided and maintained, (6) the milk utensils and cream separator be maintained in a clean sanitary condition. H.P.

409. New Dairy Products Developed by Necessities of War. MEL PRATT. Canad. Dairy and Ice Cream Jour., *25*, 9: 37. September, 1946.

A palatable new butter known as "Butter concentrated hardened" has been developed for use in tropical conditions. At a temperature of 105° F. it can be spread on bread as ordinary butter would be. It consists of dry butterfat containing 3 per cent hydrogenated peanut oil, 4 per cent skim milk powder, 2 per cent salt, and 0.2 p.p.m. diacetyl. A new cheese known as homo cheese was developed that kept for six days at 145° F. Dry butterfat was manufactured in great quantities and kept well for 12 months or more. "Tropical Spread" was developed for the army for use in tropical areas. It contained 1% salt, 2% spray dried skim milk powder, 77% dry butterfat and 20% hydrogenated butterfat. Peanut oil was later substituted for the hardening agent when it became available. Other developments are the modification of the phosphatase test to cheese, the method of packaging cheddar cheese in valve-vented cans, the packing of cheese in transparent moisture proof sheets producing rindless cheese, dehydrating and compressing cheddar cheese and army cheese spread. H.P.

410. The Use of Indicator Paper to Determine the pH of Butter. A. H. WHITE. Canad. Dairy and Ice Cream Jour., *25*, 8: 23. August, 1946.

The pH values of the cream, buttermilk and butter serum as shown by the indicator paper were lower than the pH of the butter serum by the glass electrode. As the pH of butter serum increased, the variation between the values obtained by the paper and the glass electrode became greater. Dilution of the butter serum with distilled water increased the pH as determined by the indicator paper and glass electrode. The data indicate that pH indicator papers do not give reliable pH values for cream, buttermilk, or butter serum when compared to the pH of butter serum by the glass electrode. Creamerymen should base their neutralizing practices on an accurate titration test for acidity rather than on pH values determined by an indicator paper. H.P.

411. Dosage de la Matière Grasse des Cremès. (Determination of the Fat Content of Creams.) PAUL CODOR AND PIERRE MACHEREL. Le Lait, *21,* No. 237–238: 196, and No. 239–240: 289. 1944.

All rapid tests for fat in cream utilizing a 5-cc. sample must take into account that the final butyrometer reading must be corrected because the cream density varies with its fat content. Acidity also influences the weight of sample taken. This problem is treated mathematically with reference to two types of butyrometers. The calibration of glassware is reported on and an improved mechanism for reading tests is illustrated and described. O.R.I.

412. The Importance of Volume in the Creamery Business. P. E. REED. Canad. Dairy and Ice Cream Jour., *25,* 5: 38. July, 1946.

Large creamery unit production reduces cost and invariably turns out a better quality of butter. Better equipment and closer supervision are maintained in larger plants. H.P.

CHEESE

413. Control of Insects in Cheese and Butter Factories. R. W. THOMPSON. Canad. Dairy and Ice Cream Jour., *25,* 5: 27. May, 1946.

Several insects found in creameries and cheese factories became sufficiently abundant to necessitate control measures. These include the common housefly, cockroaches, cheese mites, cheese skippers, larder beetles, and their larvae. Control measures to take on flies are (1) to prevent breeding, (2) to prevent spillage of milk or whey, (3) to keep the flies out of the building itself, (4) destroy flies that get in the building daily with DDT. The German cockroach occurs in some Canadian creameries. They can be controlled by use of a sodium fluoride-pyrethrum powder mixture as a dust with repeated treatments. Cheese mites and cheese skippers are the two worst pests found in cheese. Cleanliness is of great importance in keeping mites under control. Hot water (over 150° F.) and formaldehyde are effective in killing mites. Low-temperature storage is an excellent safeguard against mite damage. Fumigation with methyl bromide in conjunction with carbon dioxide is satisfactory in an airtight chamber above 60° F. for 24 hours. The cheese skipper attacks cheese, ham, bacon, and beef. The adults may be excluded from storage by a 30-mesh wire screen, but the low temperature controls given for mites are the most effective since reproduction cannot continue at temperatures below 56° F. Fumigation is also effective (as for mites). Larder beetles can be controlled by fumigation if heavily infested. H.P.

414. Influénce de L'Elimination de la Croute dans L'Analyse des Fromages. (Influence of Eliminating the Rind in the Analysis of Cheese.) JEAN PIEN. Le Lait, *21*, No. 237–238: 210. 1944

The composition of the rind of cheese differs from the interior of the cheese either because of loss of moisture or the action of molds, yeasts, and proteolytic bacteria during ripening. Moisture and fat determinations were made of the rind and of the interior of several types of cheese while fresh as well as partially and fully ripened. Camembert, Brie, Port Salut, and Edam were included in the study. The percentage of fat in the water-free substance was calculated in each case.

The rind of the soft cheese was sometimes drier than the interior when the cheese was new and was always so when the cheese was cured. The fat in the water-free substance was lower in the rind of soft, young cheeses due to the action of molds, but became higher than the interior after aging. This is apparently due to the more rapid loss of volatile nitrogenous matter from the rind than from the interior.

In the case of pressed cheeses such as Edam, the rind is always drier than the interior and becomes more so as ripening progresses. In the majority of cases the fat in the water-free substance is slightly higher in the rind than in the interior.

Results were compared in cases where the rind was eliminated and where it was included. For fresh and semi-cured soft cheese no regular effect was noted, but in the case of fully cured soft cheese eliminating the rind resulted in higher moisture values and lower values for fat in the water-free substance. In the case of the harder cheeses, the moisture values were much higher where the rind was eliminated. The effect of this procedure on the values for fat in the water-free substance was variable. O.R.I.

415. Les Bases Techniques de la Définition Légale et de la Fabrication des Fromages. (The Technical Bases for the Legal Definition and the Manufacture of Cheese.) JEAN PIEN. Le Lait, *24*, No. 241–243: 1. January–March, 1945.

A series of 29 formuluae are given, together with examples, illustrating how one may calculate the fat, solids, solids-not-fat, and the percentage recovery of the constituents in cheesemaking providing the requisite analytical data are at hand. Of particular interest is the "coefficient K" which indicates the weight of solids-not-fat recovered in the cheese per liter of mixed milk used. This coefficient is indicative of plant efficiency. It is suggested that the various formulae will be of value in establishing normal yields and for price fixing purposes. O.R.I.

416. Influence of Temperature on the Curing of Cheddar Cheese. H. C. Hansen. Canad. Dairy and Ice Cream Jour., *25*, 6: 48. June, 1946.

Raising the cheese ripening temperature from 40° F. to 60° F. increased the loss of weight from 4.44% to 8.71% during six months storage. Cheddar cheese ripened at 40° F. for 6 months was usually criticized for being slightly acid, lacking in flavor or flat. Cheese ripened at 50° F. was high acid, bitter, or very slightly fruity. Cheese cured at 60° F. was high acid, sharp or slightly fruity. Cheddar cheese made from good milk developed more flavor in three months at 60° F. than did cheese cured at 40° F. and 50° F. for 6 months. Cheddar cheese made from good quality raw milk developed more flavor in a shorter time than did cheese made from pasteurized milk, but it was not as uniform in flavor. H.P.

417. Effect of Varying Amounts of Salt on the Quality of Cheddar Cheese. E. B. Tustin, Jr., Worcester Salt Co., Natl. Butter and Cheese Jour., *37*, 9: 44. September, 1946.

Weak, pasty body, open texture, abnormal ripening, and greater shrinkage during curing are all associated with under-salting of cheese. Oversalting causes dry, harsh body, cracking of the cheese rind, excessive mold damage, and discoloration in colored cheese. Salt should be added in small quantities and thoroughly stirred between each addition. Holding salted curd in the vat for about 20 minutes before hooping is desirable. For every 1,000 pounds of milk containing approximately 3.5% fat there should be added to the curd 1 pound of salt for every 1% of salt desired in the curd. Variations above or below this proportion must be made to conform to the cheese-yielding capacity of the milk and quality of the curd. It is never advisable to add less than 2% salt to curd; 2½% is most satisfactory for normal curd. W.V.P.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

418. L'analyse Chimique des Colles en Poudre à Base de Caseine. (Chemical Analysis of Casein-Based, Powdered Glues.) J. Pien and G. Renvoise. Le Lait, *25*, 244–246: 121. 1945.

Casein glues vary greatly in composition and properties. In addition to casein, such materials as gelatin, dextrin, chalk, calcium and sodium carbonates, sodium fluoride, sodium silicate, and many other inorganic substances may be present. Methods of analyses are given which include separation of organic and inorganic materials, as well as the qualitative and quantitative methods for these. A convenient and fairly accurate method of separating constituents consists of shaking 10 grams of powdered glue in 100 ml. of chloroform. The organic matter can then be decanted. If gelatin is present it will dissolve in tepid water. Dextrin can be detected by the iodine reaction. Recognized methods of inorganic analysis are employed for further elucidation. Examples are given showing the accuracy of the method when applied to samples of known composition.

O.R.I.

DISEASE

419. The Efficacy of the Microscope Examination of the Incubated Producer Milk Samples in Detecting Streptococcic Mastitis in Dairy Herds.

H. P. JOHNSON, City Health Dept., Lansing, Mich., AND C. S. BRYAN, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., *9*, No. 4: 197–201: 248. July–August, 1946.

Individual samples of milk were checked for streptococcic mastitis on 863 cows in 83 herds. The examination showed 78.3 per cent of the herds and 21.9 per cent of the cows were infected.

Producer samples were also collected on the same day from each of the dairy herds and examined microscopically, following incubation. The results are summarized as follows:

1. In 60 herds a positive producer sample showed accurately the presence of streptococcic mastitis, as shown by the examination of individual cow samples from each herd.
2. In 4 herds positive producer sample results were not verified on examination of cow samples. The possibility of contamination from previous infected samples is suggested, since they were taken from the weigh tank. If the producer samples were collected from the cows, this contamination may be reduced to a minimum.
3. In 5 herds a negative producer was not confirmed upon examination of the individual cow samples, because in 4 herds one infected cow was found and in the fifth herd two were located. No explanation is offered for these results.
4. The remaining 14 herds negative producer samples showed freedom from infection as confirmed by the results of individual cow testing.

H.H.W.

FEEDS AND FEEDING

420. **Milking for Profit Requires Good Pasture, Feed and Care.** D. H. McCallum. Canad. Dairy and Ice Cream Jour., *25*, 7: 38. July, 1946.

Two methods of increasing returns are (1) to receive a higher price for the commodity produced and (2) to reduce the cost of production. To increase profit it is necessary to increase production per cow, selection and weeding through cow testing, better use of pastures, wise grain feeding, more efficient use of labor, prevention of disease, and producing a good quality product. H.P.

421. **Dried Whey as a Substitute for Dried Buttermilk in Chick Rations.** S. J. Slinger, F. N. Marcellus, and E. V. Evans, Ont. Agr. College, Guelph, Ontario. Sci. Agr. *26*, No. 8: 381-387. August, 1946.

"Dried Whey" in amounts up to 8 to 10 per cent was used satisfactorily in rations for growing chicks to replace part of the dried buttermilk when the necessary adjustments were made in the riboflavin and protein levels of the ration. Higher levels of dried whey, 12 to 20 per cent, resulted in the onset of an oedematous condition, although growth, as judged by weight and mortality, was little, if any, affected. This condition was accompanied by an increased water consumption and the external symptoms gradually disappeared.

"Although the quantity of dried buttermilk necessary for growth appears to be small, there is some indication in this work that it should not be completely replaced by dried whey. There is also a suggestion that dried whey may contain some essential growth factor or factors not present in dried buttermilk." O.R.I.

FOOD VALUE OF DAIRY PRODUCTS

422. **Les Substances Choliniques du Lait. (The Choline Substance of Milk.)** E. Kahane and Mlle. J. Levy. Le Lait, *25*, 247-248: 193-223. 1945.

An extensive study is reported of the choline content of cows' milk with some values reported for ewes' milk as well. Details are given of the analytical methods, which include treatment of the milk with ferric sulfate and calcium carbonate to produce a clear filtrate, the latter being evaporated to dryness and then redissolved quantitatively in water. Free choline is then determined by the disappearance of the Florence reaction (microscopic observation for crystals of choline periodide).

In addition to free choline the filtrate contains a substance or group of substances distinct from the phosphoaminolipids which furnish an acid-hydrolyzable choline. This is determined on an aliquot of the filtrate by refluxing with concentrated hydrochloric acid. Extraction of the total choline is done by treating the original milk sample with 65% sulfuric acid for 24 hours at 105° C. Non-hydrolyzable choline was determined by differences.

Average results on 100 samples of cows' milk showed choline present in the form of free choline and hydrolyzable combined choline at from 15 to 35 mgm./l. Variations could not be correlated with such factors as season, feed, breed, age or stage of lactation except that free choline was greatly reduced at calving.

Values for total choline were much more constant ranging between 160 and 280 mgm./l. with an average of 222 mgm. Milk enzymes are apparently inactive with respect to the conversion of choline from one form to another.

There appeared to be no close relationship between choline and the fat globules, choline being largely recovered in the skim milk fraction and equally as rich in the fore-milk as the strippings. Sheeps' milk contained somewhat less free and total choline than did cows' milk. O.R.I.

ICE CREAM

423. **Scientists Develop New Sugar.** ANONYMOUS, Ice Cream Field, *48*, 3: 35. September, 1946.

A method of extracting levulose—a sugar 50 per cent sweeter than sucrose—has been developed by the University of Colorado Engineering Experiment Station at Boulder, Colorado, according to an announcement by Dr. Robert C. Hackett, Scientific Director of the Sugar Research Foundation. The method was developed by M. M. Reynolds, Junior Research Engineer, while working with Dr. Carl W. Borgmann, Director of the University of Colorado Experiment Station, and is sponsored by the Sugar Research Foundation.

Briefly the process is as follows: Sucrose is hydrolyzed by acid and the levulose precipitated with lime. Then after filtering, the lime is precipitated by bubbling carbon dioxide through the solution and freeing levulose which then dissolves. After concentrating the levulose by evaporating part of the water the sugar is crystallized in a hand ice cream freezer during slow stirring.

It is stated that about 100 pounds of levulose has been made by this process. Expectations are that additional studies will be made to estimate costs and solve technical manufacturing problems. W.C.C.

424. Strong Arm Dipping—Improper Cabinet Temperatures Cause Heavy Loss. ANONYMOUS, Ice Cream Field, *48*, 2: 30. August, 1946.

The importance of controlling ice cream dipping temperatures is emphasized. It is stated that temperatures below 10° F. or above 16° F. will result in loss of time or overrun. It is mentioned, however, that authorities do not agree on the optimum dipping temperatures—some prefer 10 to 12° F. while others claim 5 to 8° F. give best results. W.C.C.

425. Storing Ice Cream Ingredients for Short Supply Use. R. J. RAMSEY, Canad. Dairy and Ice Cream Jour., *25*, 9: 46. September, 1946.

It is necessary to store butterfat and milk solids-not-fat because of fluctuation in seasonal production of milk. The following products are discussed, with the best method of manufacture and storage listed: (1) frozen cream, (2) plastic cream, (3) butter, (4) butteroil, (5) sweetened condensed skim milk, (6) frozen plain, (7) condensed milk, (8) spray skim milk powder, (9) roller process powder. H.P.

426. Treating Nuts for Ice Cream. C. A. IVERSON, Head, Dairy Department, Iowa State College, Ames, Iowa. Ice Cream Field, *48*, 2: 22. August, 1946.

Recipes are given for making buttered pecan, English walnut, black walnut, hazelnut, cashew, or pinon or pignolia, and pistachio. All recipes are similar to the one for buttered pecan which follows:

Dry roast 8 pounds pecans until brown throughout the nut, then cool quickly. Heat 1½ pounds of butter to 330° F. (160° C.). Add roasted nuts and one ounce of salt. After few minutes drain off butter. Add 5 pounds buttered nuts to 10 gallons of finished ice cream. W.C.C.

MILK

427. Cost of Producing Milk. L. C. CUNNINGHAM, Professor of Farm Management, Department of Agricultural Economics, New York Agricultural Experiment Station. Ice Cream Field, *48*, 3: 46. September, 1946.

The author gives results of cost studies made in various parts of the State of New York. He states that the average feed and labor requirements per 100 pounds of milk equal 34 pounds of grain, 77 pounds of hay, 114 pounds of silage and 2.6 hours of man labor. Based on $75.00 a ton for dairy ration, $15.00 a ton for hay, $6.00 a ton for silage and $.55 per hour for labor, the cost of producing 100 pounds of milk is estimated by formula to be $4.35. If the labor cost were $.65 per hour it would amount to $4.61 per hundredweight.

It is also pointed out that the change in actual cost of $2.07 per hundredweight in 1939–40 to the current, estimated cost by formula of $4.35 represents an increase of 114 per cent.

The author states "Although it is cheaper to produce milk in the pasture season than it is in the winter season, it does not follow that the year-round cost of producing 100 pounds of milk is lower in summer dairies than in winter dairies." This indicates that the reverse is often true.

The author concludes,

"High production per man was obtained mainly by these practices: (1) keeping moderately large herds which made possible (2) spending less than the average amount of time per cow to do the chores in the stable, yet (3) having better-than-average producing cows." W.C.C.

428. Un Nouveau Butyromètre Pour L'Application de la Méthode Gottlieb-Röse. (A New Butyrometer for Application of the Röse-Gottlieb Method.) Jean Pien, Laboratories de la Laiterie des Fermiers Reunis. Le Lait, *24*, No. 239–240: 341. October–December, 1944.

A special extraction flask claimed to be superior to the Rieter and Rohrig tubes is illustrated and described. The flask is a straight, glass-stoppered tube with expanded portions at the base, center and top and cubic centimeter graduations on the upper and lower necks. Immediately above the lower graduation is a side-arm and glass stopcock. A 10-ml. sample of milk is used. The flask is claimed to permit easier and more accurate separation of the ether fraction. O.R.I.

429. Le Calcul de L'Extrait Sec du Lait. (Calculation of the Total Solids Content of Milk.) E. Muller-Hoessli, Laboratoire Contonal de Schaffhouse. Le Lait, *24*, No. 239–240: 310. October–December, 1944.

Halenke and Moslinger have suggested the following formula for estimating the total solids content of milk.

$$\text{T. S.} = \frac{(\text{Fat} \times 5) + \text{Density}}{4}$$

This gives results slightly lower than the Fleischman formula and the author proposes the following modification of the Halenke-Moslinger formula:

$$\text{T. S.} = \frac{(\text{Fat} \times 4.8) + \text{Density}}{4} + 0.26$$

Results on 27 samples indicate that this modification gives values very similar to those obtained by the Fleischman formula. (The values used as densities are apparently Quevenne lactometer values.) O.R.I.

430. Causes of Variations in the Butterfat Content of Milk. V. E. GRAHAM. Canad. Dairy and Ice Cream Jour., *25*, 6: 34. June, 1946.

The factors operating over long periods are: (1) age of cow, (2) influence of season, (3) influence of stage of lactation, (4) combination of long time factors. The factors causing short-time variations are: (1) night and morning milk, (2) temperature, (3) excitement, (4) fatness of the cow at calving time. The author states that (1) milk varies in fat test to a greater extent than is generally realized, (2) any variation in the fat test of the milk will cause a change many times as great in the fat test of the cream separated from it, (3) morning milk usually has a lower fat test than evening milk, (4) excitement and sudden changes in temperature are common causes of sudden variations in fat test, (5) if most of the cows freshen in the spring, the lower test due to the seasonal factor will coincide with the lower test due to the stage of lactation, (6) feed has a marked influence upon the color of milk but the fat test of milk cannot be consistently increased by the use of specific feeds. H.P.

431. Research on Rancidity in Milk Greatly Advanced Since 1726. W. L. DUNKLEY. Canad. Dairy and Ice Cream Jour., *25*, 6: 27. 1946.

A bitter flavor described by Lawrence in 1726 was probably a form of rancidity. Rancidity goes by a number of different names such as bitter rancid, goaty or wintry flavor. In a milk form it is also frequently described as an unclean or cowy flavor. The bitter rancid flavor always develops after milking. Twenty years ago investigators tried to explain the defect as of bacterial origin. It is now generally agreed that lipase causes rancidity. The best general recommendations for the control of rancidity are (1) feed the cows a ration of good quality, (2) do not subject the milk or cream to vigorous agitation nor to temperature fluctuations during storage, and (3) ship regularly. H.P.

432. Raw Milk Contamination in Homogenized Milk. A. V. MOORE AND G. M. TROUT. Canad. Dairy and Ice Cream Jour., *25*, 7: 33. July, 1946.

Observations with respect to development of rancidity, titratable acidity, pH and phosphatase tests were made in five series of homogenized and non-homogenized milk to which various increments of raw milk had been added. During storage at 40° F. raw-milk-contaminated homogenized milk increased in titratable acidity, decreased in pH and developed rancidity. As little as 0.5% raw milk could be detected by the phosphatase test, questionably when 0.1% was added, but not when 0.05% was added. At 24 hours storage contamination of homogenized milk with raw milk must be at least 4% before rancidity can be detected by taste and smell. Extracting the phenol with butyl alcohol should be a required step and not optional in the phosphatase test, particularly when applied to homogenized milk.

H.P.

433. Use of Composite Milk Samples for Making Sediment Tests. E. G. Hood and I. Hlynka. Canad. Dairy and Ice Cream Jour., *25*, 9: 27. September, 1946.

The sediment test consists of filtering a definite quantity of milk through 1½ inch linten filter-discs. The sediment present is an indication of the care used in handling or producing milk on the farm. The use of the sediment test can be greatly extended among cheese-factory patrons provided the test is made on composite samples of milk so as to reduce the amount of work. The test is carried out as for regular composite samples only for an Imperial pint, three ⅓-oz. dippers of milk are required daily over a period of 13 days, or for a U. S. pint, 2 dippers for 14 days would give an adequate amount of sample. The samples are heated to 70° F. or above before making the test. The sample is inverted two or three times and transferred to the sediment tester. The bottle is half-filled with hot water 130–140° F., rinsed, and the rinse water passed through the same disc. H.P.

434. Significance of Concentration of Coliform Organisms in Raw Milk upon Survival of Pasteurization. J. E. Craige, Veterinary Section, Ninth Service Command Medical Laboratory, Presidio of Monterey, California. Jour. Milk Technol., *9*, No. 4: 191–196. July–August, 1946.

Eight strains of Escherichia-Aerobacter organisms were isolated from raw milk, pasteurized milk and ice cream, and were able to survive in milk at 62° C. for 30 minutes when present in sufficient number. None of these microorganisms survived when the concentration before pasteurization was less than 700 per ml.

Pasteurized milk should not be considered satisfactory for human consumption if the milk is positive for coliform organisms in more than two of five 1-ml. samples.

When coliforms are persistently present in the pasteurized milk, the causes may be due to (1) contamination after pasteurization, (2) inadequate pasteurization, (3) improper handling of the milk after pasteurization, (4) excessive numbers of coliform organisms in raw milk, and (5) heat-resistant strains.

The use of the phosphatase test, the standard plate count, the coliform test, laboratory pasteurization of raw milk, and other special techniques, in addition to line samples taken at the plant, are recommended procedures in order to determine the specific cause of contaminations that are difficult to control. H.H.W.

435. Le Controle de la Pasteurisation du Lait et de la Crème. (The Control of Pasteurization of Milk and Cream.) JEAN PIEN. Le Lait, *25,* No. 249–250: 310. 1945.

Tests for the presence of peroxidase in milk, cream, and butter have long been used as indicators of the effectiveness of pasteurization. Such methods as those of Dupouy (guaiacol), Storch (paraphenylenediamine) and Rothenfusser (a combination of the two previous tests) all lack reliability in cases where iron or copper are contaminants. The Schardinger reaction (a test for the presence of aldehyde dehydrogenase) is not affected by metals. In it, 10 ml. of milk to be tested is allowed to react at 50° C. with 0.5 ml. of an aqueous solution containing methylene blue and formaldehyde.

The results of a number of parallel trials are presented in which milk samples were heated to temperatures ranging from 72 to 83° C. for periods ranging from less than 10 seconds to more than 30 minutes. The results indicate that the Schardinger enzyme is slightly more sensitive to heat than is peroxidase when the latter enzyme is tested for by the Dupouy test. If the Schardinger reaction is negative after 4 hours incubation, proper pasteurization is indicated. It is suggested that this test is preferable for cream. O.R.I.

436. Antagonisme du Colibacille et des Bacteries Padrides Dans le Lait Contaminé. (Antagonism of Coliforms and of Putrefying Bacteria in Contaminated Milk.) A. ROCHAIX AND F. SIMON. Le Lait, *25,* 244–246: 144. 1945.

The authors have made a comparison of the relative growth of coliform organisms as against putrefying bacteria, the latter consisting of types capable of liberating hydrogen sulfide and of producing black colonies in a lead acetate medium. It is shown that a marked antagonism exists between the two types, in some cases either type completely inhibiting the growth of the other. The mechanism of antagonism is apparently not due to the high acidities or free hydrogen sulfide but to more complex antibiotic agents.

O.R.I.

437. Milk for Manufacturing Must Be of Top Quality. J. C. HENING. Canad. Dairy and Ice Cream Jour., *25,* 9: 43. September, 1946.

Milk for the army was rejected if it did not attain the high standards set according to recognized standard tests. The methylene blue reduction time, direct count, plate count, and sediment testing were used as the standard tests. The Quartermaster Food and Container Institute had mobile laboratories for making chemical and bacteriological tests at canning plants. Dairy plants making dry whole milk, dry non-fat milk solids, and dry ice cream mix also used these mobile laboratories. The tests run on the farmers' milk and composite samples of milk were resazurin, methylene blue, standard plate count, direct count, thermophiles, coliforms, and anaerobes. Within the plant the bacteriological tests made were standard plate count, direct count, thermophiles, coliforms, and anaerobes. Samples for these tests were taken after separation, after standardization, after preheating, and prior to drying. Other tests determined were acidity, pH, oxidation-reduction potential, copper, ascorbic acid, titration to a pH of 4.63, and conductivity. H.P.

438. The Responsibility and Work of Milk Sanitarians. C. C. HADLEY. Canad. Dairy and Ice Cream Jour., *25*, 9: 30. September, 1946.

A milk sanitarian is a person making a profession of the application of sanitary and health measures to the production, processing and distribution of milk and milk products for the public benefit. The training for employment as a milk sanitarian should be somewhat broader than those provided by the usual dairy curriculum. There is no line of demarcation between the primary interests of the producer, processor and the sanitarian in the production of high quality, sanitary milk, and milk products. H.P.

439. Types of Refrigeration for Market Milk Plants. A. A. GEIGER. Canad. Dairy and Ice Cream Jour., *25*, 5: 42. July, 1946.

The refrigeration should be designed to have sufficient flexibility to easily handle the maximum load and still require no hand regulation of control valves for minimum loads. The three types of refrigeration are brine, direct expansion of refrigerant, and sweet water. The single aim for each system is to store up refrigeration during the night to be used the following day. Ice bank storage is not conducive to the processing of good milk. For a small plant a cabinet type water cooler or a brine cooling system can be used. Refrigeration requirements are figured for a plant bottling around 1500 gallons per day. Brine system is adaptable to a plant of this size better than an instantaneous water cooler. Direct expansion is not advisable for use in the majority of small plants. The major reasons for this are: (1) the cost is not justified on small volumes, (2) complicated controls, (3) this system is more subject to short-cycling of compressors operation, (4) it is expensive. H.P.

PHYSIOLOGY

440. Blood Levels of Calcium and Inorganic Phosphorus in Hereford Cattle. MERLE G. PAYNE, A. S. CLARK, H. E. KINGMAN, AND W. M. STANBURY. Jour. Agr. Res., *72:* 12. June 15, 1946.

The normal blood levels of phosphorus and calcium for range Hereford yearling and herd bulls and for range Hereford heifers and aged cows was determined on 560 arrivals. The normal levels for blood serum inorganic phosphorus in milligram per cent were 7.30 ± 0.103, 4.76 ± 0.099, 5.07 ± 0.063, and 4.89 ± 0.101 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively. The phosphorus level of blood was found to be greater in bulls than in cows. The quantity of phosphorus diminishes with age in Hereford range cattle, but this relationship with age is stronger in the male animal. The normal levels of blood calcium in milligram per cent were 10.46 ± 0.238, 13.03 ± 0.256, 9.13 ± 0.158, and 9.52 ± 0.249 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively. A complementary association of phosphorus and calcium has been found. The calcium-phosphorus ratio in homogeneous groups is a variable concept depending on age and sex. H.P.

441. The Component Acids of Cow Colostrum Fat. C. P. Anantakrishnan, V. R. Bhalerao, and T. M. Paul, Imperial Dairy Research Institute, Bangalore (India). Biochem. Jour., *40,* No. 2: 292–297. 1946.

The colostrum was collected from six different cows, representing four different breeds of India cattle. It was then diluted with water and the cream separated. The butterfat was then prepared by the "boiling off" method. Tables giving the more common physical constants of the individual samples, as well as a composite mixture, for the first to fifth, tenth, and fifteenth days of lactation, are shown. The mixed composite samples for each day were converted to methyl esters and fractionated. The transition from colostrum to normal milk was accompanied by an increase in the saponification and Reichert values and a decrease in the butyro-refractometer reading, the iodine values, and the melting point. "The first day's cow colostrum fat compares favorably with the mature human milk fat, the percentage of lower acids up to C_{14} being almost the same (16.4 and 17.8, respectively)." A.O.C.

442. Le Colostrum de Vache. (Cow's Colostrum). A. Houdinière. Le Lait, *24,* No. 234–236: 108, No. 237–238: 226, No. 239–240: 313, 1944; *25,* No. 241–243: 27, No. 244–246: 97, 1945.

The changing properties of colostrum are fully reviewed, French, German, English, and American literature all being reported. Under physical properties, freezing point, specific gravity, viscosity, surface tension, index of refraction, and electrical resistance are dealt with. The increased content of albumin, globulin, and soluble phosphates influence creaming, heat coagulation, titratable acidity, and pH. Acid precipitation of the casein is interfered with because of the high level of albumin and globulin. Colostrum contains large numbers of cells, many of which appear to be of blood origin. By classifying the cellular material it is possible to determine whether the colostrum is from a healthy or infected udder. Phagocytes are present in colostrum and its bactericidal properties are more marked than those of fresh milk. It is particularly rich in opsonins and contains agglutinins, precipitins and lytic antibodies for some types of organisms. The antibiotic properties of colostrum should be studied in relation to mastitis organisms, the author states.

Catalase activity is 10 to 12 times greater than in milk; lipase and amylase activities are greater, but Schardinger enzyme, protease, and phosphatase activities are less. Among the vitamins, the data available indicate that most of these factors are richer in colostrum. The saffron-yellow color is due mainly to carotene and riboflavin.

The energy value of colostrum is upwards of twice that of milk. Digestibility and action of rennet are discussed extensively, as is the physiology of secretion. The author points out that colostrum was probably the major product of the mammary gland until man began to select and breed animals for more persistent milk production.

The extent to which colostrum may be present in a milk supply can be determined from the relative proportion of "holoproteins" (albumin and globulin) to the total protein. The various effects which the presence of colostrum might have upon almost all types of dairy manufacturing are fully but somewhat speculatively discussed on the basis of the properties of colostrum. 273 references are cited. O.R.I.

443. La Genotypicité des Enzymes Microbiens Illustrée par les Ferments Lactiques. (Specific Genotypes of Microbial Enzymes Illustrated by Lactic Ferments.)

Costantino Gorini. Le Lait, *24*, No. 237–238. July–September, 1944.

Production of enzymes by bacteria affords a means of establishing classifications. The author claims that bacterial species display a similarity to other biological genotypes in that descendants are similar to parents, yet at the same time individual cells vary from type somewhat. Dissociation in the bacterial cell increases this tendency toward variation because it aids a bacterial species in adapting itself to new media. The work of several bacteriologists is cited to show that, in the case of the lactic acid bacteria, recognition of this adaptive faculty has resulted in unifying several sub-species of organisms into one species. O.R.I.

MISCELLANEOUS

444. Values of Cleaners in the Dairy Industry.

V. E. Graham and G. E. Turner. Canad. Dairy and Ice Cream Jour., *25*, 7 : 27 ; *25*, 8 : 36. July–August, 1946.

The residues to be removed from dairy equipment consist of milk fat, milk proteins, and the salts of milk, together with those salts which are formed as a result of chemical combination between the salts of the water and milk solids. The fat and most of the milk proteins are not soluble in water. Commercial cleaners usually remove dirt by one or more of the following procedures, namely: solution, emulsification, wetting, deflocculation and foaming. The washing problem is simplified when dairy plants have a soft-water supply. Hard water causes precipitates with some cleaners and influences milkstone formation. Any compound to be valuable as a cleaner must possess one or more of the following qualities: (1) wetting power, (2) emulsifying power, (3) deflocculating power, (4) dissolving power, (5) water-softening power, (6) free rinsing, (7) inert towards metals, (8) cost. The advantages and disadvantages of the common alkaline cleaners are considered. The ones mentioned are sodium hydroxide, sodium carbonate, trisodium phosphate and sodium metasilicate.

Milkstone removers are nearly all acid substances or mixtures of acids. The common acids used are hydrochloric, tartaric gluconic, or citric acid. New ideas in cleaners eliminate precipitates with hard water and increase wetting power. Complex sodium phosphates are satisfactory as they do not form precipitates with hard water, are non-corrosive, prevent milkstone formation, and have good wetting powers. Most wetting agents are sulphonated alcohols, sulphonated esters and sulphonated ethers. Special cleaning directions are given for each piece of equipment, including receiving and storing equipment, can washing, bottle washing, preheating equipment, plate heaters, churns, floors, walls and painted surfaces, aluminum equipment, glass enamel, and stainless steel. H.P.

445. Softening and Purifying Water for Use in Dairy Plants. C. E. LENNOX. Canad. Dairy and Ice Cream Jour., *25,* 7: 35. July, 1946.

In getting a plant water supply one must take into consideration: (1) source of water, (2) sources of impurities, (3) suspended water, (4) taste, odor, and color, (5) hardness, (6) iron, (7) bacteria. Notable hard-water troubles in the dairy are (1) boiler operation, (2) hot-water supplies, (3) can washing, (4) bottle washing, (5) water-cooling coils, (6) equipment cleaning, (7) washroom maintenance. Several processes or types of water softeners are available: (1) hot process lime-soda, (2) cold process lime-soda, (3) siliceous zeolites, (4) carbon-type exchangers. H.P.

446. Seeing Is Believing in Sanitary Control. M. C. JAMIESON, K. H. CHEN, AND D. A. WILLIGAN. Canad. Dairy and Ice Cream Jour., *25,* 7: 52. July, 1946.

A simple procedure for assisting eating and drinking establishment personnel in sanitation problems involves a suitcase type of laboratory based on the swab-streak principle. The procedure from swabbing of the surfaces to the examination of the results is carried out in the establishment. Sufficient detail is presented to enable anyone with access to a bacteriological laboratory for the preparation of supplies to apply the method in any educational or control program. H.P.

447. Freon and Ammonia Mechanical Refrigerants. T. M. HUGHEY. Canad. Dairy and Ice Cream Jour., *25*, 6: 31. June, 1946.

In the past few years several refrigerants have been utilized and new ones developed. Ammonia has been used since mechanical refrigeration was invented. The selection of a refrigerant depends upon the type of machine or system in which it is to be used. The ASRE Data Book lists approximately 30 refrigerants. During the war different Freon liquids were shown to be good refrigerants using much lighter equipment and suitable for heavy and for lighter jobs where leakage would not spoil foods. For maximum economy, ammonia still holds its place in the industrial field. On small and fractional tonnage application, its use is diminishing. Operation costs per ton, both theoretical and practical, are lower for ammonia than any other refrigerant used in industrial systems. The properties of ammonia are well established. Freon as a group has many desirable properties such as (1) lower pressures, (2) constructed of lighter materials, (3) it is an active cleaning agent, (4) it is not miscible with water, (5) it is odorless and non-toxic, (6) it is a very stable refrigerant. H.P.

AUTHOR INDEX OF ABSTRACTS

ABBOT, FRED H., why color of butter is standardized, A31

Abdel-Malek, Y., formation of carbon dioxide bacteria, A88

Abele, C. A., methylene-blue test, A60

Ackerman, H. A., post war plant operation, A12

Adams, Thurston M., wartime changes in milk distribution, A28

Alter, E. I., installation of refrigerating equipment, A48

American Society of Refrigerating Engineers, the refrigerating data book, 1946, A169

Ammel, T. J., freon refrigerating systems, A50

Anantakrishnan, C. P., component acids of colostrum fat, A204

Anderson, C. W., control of mastitis, A36

Anderson, E. B., the resazurin and methylene-blue tests, A100

Anderson, E. O., mastitis and the plate count, A56

Anderson, J. Ansel, enzymes and their role in wheat technology, A162

Andrews, G., adsorption of pepsin and rennin on foam, A118

Anglada, J. Alfred, tomorrow's packages, A187

Anonymous, lactic acid from whey, A4; acceptance of dried milk, A5; anti anemia factors in milk, A6; use of ascorbic acid to blanch fruits, A9; first American ice cream, A9; Yami Yogurt, A19; processed cream, A19; trend toward bigger plants, A26; frozen foods, A27; costs, A38; the fountain of tomorrow, A38; ice cream sales, A39; food chains sell ice cream, A39; food from casein, A56; fatty acids in butterfat, A58; dairy farm methods, A62; sonic-soft-curd vitamin D milk, A103; bulk gallon sales, A124; penicillin ice cream, A126; new plant construction, A126; opportunities in retail trade, A134; continuous butter churn, A163; diagnosis of brucellosis, A176; vitamin C in milk products, A178; roadside ice cream business, A179; new sugar, A196; cabinet temperatures, A198

Arbuckle, W. S., estimation of salt in butter, A143

Archibald, J. G., chemistry of grass silage, A121

Armstrong, T. V., cleaning of square and round milk bottles, A102; cleansing of square and round milk bottles, A133

Asdell, S. A., induce heat in the ovariectomized cow, A65

Atkinson, Ida, ascorbic acid peach pack, A77

BAILEY, A. E., flavor reversion in edible fats, A68; industrial oil and fat products, A73

Bailey, Katherine B., home freezers, A81

Baratte, J., vitamin pp content of cow's milk, A123

Barnicot, C. R., reactions and properties of annatto, A93

Bartlett, Rolland W., milk distribution cost, A132

Baslet, Fred C., fiber milk containers, A181

Beach, B. A., bactericidal activity of bovine serum, A70

Beau, Maurice, is "casein" of soya bean casein? A119

Beckel, A. C., soybean or vegetable milk, A72

Beezley, Margaret B., determination of thyroxine in iodinated casein, A134

Bendixen, H. A., stabilizers in ice cream, A7; the butter industry, A116

Berard, H. L., bacterial content of egg melange, A97

Berridge, N. J., purification and crystallization of rennin, A33

Bertonasco, E. T., manufacture of parmesan cheese, A69

Beyer, M. R., *Salmonella typhimurium* food infection, A178

Bird, E. W., butter from neutralized cream, A31

Birdseye, Clarence, preservation of foods by freezing, A83

Bischoff, Fritz, biologic response to the gonadotrophins and to insulin, A72

Blair, Howard A., field refrigeration installations, A185

Blanford, C. J., production problems, A17

Bloor, W. R., new concept about butter, A89; new concept about butter, A142

Blumenthal, S. K., chocolate outlook, A129

Bohn, J. D., design of milk houses, A18

Bohstedt, G., value of whey as a food, A145

Bolin, D. W., vitamin A potency of butter, A2

Botwright, W. E., a new germicide, A158

Boutwell, R. K., utilization of galactose, A164

Bowie, D. A., resazurin and methylene blue tests, A151

Brandon, J. H., Babcock test on homogenized milk, A40

Breed, Robert S., coliform bacteria in pasteurized milk, A14; inspection problems, A154

Brew, James D., the bacteria "count," A67

Bricker, George, vitamined ice cream, A75

Brody, Samuel, bionergetics and growth, A1

Brown, A. E., plastics from acylated casein, A55

Brown, J. B., acids of human milk fat, A164

Brown, R. W., shrinkage of butter, A141; buttermilk in making bread, A148; percentages of fat in cream, A154

Browne, H. H., estimation of sucrose and lactose in mixtures, A4

Bryan, C. S., dairy bacteriology and public health, A1; the mastitis problem, A35; penicillin and mastitis control, A119; detecting streptococcic mastitis, A195

Bryant, L. R., retention of nutrients in cheese, A32; milk can washing, A130; pH and its applications, A175

Bryce, W. A., sealing vacuum-packed tins, A20; dried milk powder, A149; dried milk powder, A149; dried milk powder, A149

Buchbinder, Leon, coliform studies of pasteurized milk, A182

Buehler, Leon, Jr., plant refrigeration, A21; refrigeration in the dairy plant, A63

Bundesen, Herman N., control of the quality of milk, A133

Burgwald, L. H., cleaning of square and round milk bottles, A102; keeping quality of pasteurized milk, A106; keeping quality of milk, A107; cleansing of square and round milk bottles, A133

Burns, Robert A., "homogenized vitamin D milk," A78

Burtner, John C., condensed milk by new method, A34

Butterworth, J. F., tomorrow's packages, A188

CALHOOUN, JOEL N., field refrigeration installations, A185

Cameron, Geo. M., *Salmonella typhimurium* food infection, A178

Cannon, H. J., riboflavin determination, A34

Cass, Thomas F., Jr., tomorrow's packages, A83

Casselman, P. H., why Canadians are low cheese consumers, A117

Cathcart, Wm. H., high speed defrosting, A66

Cavanaugh, W. W., high-temperature, short-time pasteurization, A27; pasteurization by high temperature–short time, A105

Cecil, R. C., ascorbic acid peach pack, A77

Chapman, R. A., stability of whole milk powder, A150

Chen, K. H., swab-slant test for equipment, A31; sanitary control, A206

Christensen, Paul B., warehousing of frozen foods, A44

Churchill, E. S., control of microorganisms in storage rooms, A186

Clark, A. S., blood calcium and inorganic phosphorus, A203

Clunan, A. B., tomorrow's packages, A85

Codor, Paul, fat content of creams, A192

Cole, H. H., bloat in ruminants, A57

Cook, W. H., reduction of spatial temperature variations, A21; new buttermaking processes, A114

Coons, K. W., surface active agents, A171

Corash, Paul, control of milk watering, A166

Coulter, S. T., cooling cream for churning, A89; solubility of nonfat dry milk, A94; antioxidants, A148

Couturier, M. H., detecting aluminum foil coatings, A107

Cox, G. A., production of diacetyl by betacocci in milk, A88

Craige, J. E., coliform organisms, A201

Cranston, H. A., determination of traces of copper, A175

Crawford, E. A., quality control of milk, A165

Cressman, Audrey, diagnosis of bovine brucellosis, A152

Crocker, Sabin, piping handbook, fourth edition, A73

Crossley, E. L., spray-dried milk powder, A94; keeping properties of whole milk powder, A99

Cruess, W. V., nectar research, A180; studies of frozen food samples, A184; studies of frozen food samples, A187

Cunningham, L. C., cost of producing milk, A198

Curran, Harold R., activity of penicillin in preservation of milk, A173; activity of streptomycin in preservation of milk, A173

Dahlberg, A. C., keeping quality of pasteurized milk in N. Y. area, A107; survival of *Brucella abortus* in cheese, A147

Dahle, Chester D., shrinkage defect, A7; scarcity of sugar, A8; a grating type cheese, A26; how to make ice cream, A59; ice cream making, A71; new cellulose gum stabilizer, A125

Dairy Indus., Bureau of, vitamin A in butter, A25

Davis, H. P., riboflavin content of cheese, A2

Daughters, Milo R., freezing foods, A185

DeAlba, J., induce heat in the ovariectomized cow, A65

Dearden, D. V., balance of certain milk nutrients cheese, A90

Deribere, Maurice, the drying of casein, A119

Dibblee, Diana D., cationic germicides, A166

Diehl, H. C., technological supervision in the food industry, A80; technological supervision in the food industry, A84

Drew, D. E., tomorrow's packages, A79

Dubos, Rene J., the bacterial cell, A137; cationic germicides, A166

Duncan, C. W., effect of yeast and pyridoxine on poikilocytosis, A56

Dunkley, W. L., standardizing the pH of butter, A140; flavor of butter, A140; research on rancidity, A200

Duplan, J. H., a selection system for route salesmen, A131

Dyer, B. W., cane sugar, A128

Eckman, Donald P., industrial process control, A45

Edgell, J. W., keeping properties of whole milk powder, A99

Egard, A., peroxidase reaction, A155

Elvehjem, C. A., utilization of galactose, A164

Engeler, W., die haltung der zuchtstiere, A113; photographieren landwirtschaftlicher haustiere, A113; extent of inbreeding, A113

Epstein, G. A., effect of penicillin on mastitis organisms, A36

Erekson, Arthur B., cheese price, A144; extraneous matter, A174

Esmond, C. W., educating fountain clerks, A12

Espe, Dwight, secretion of milk, A111

Evans, E. V., dried whey in chick rations, A196

Evans, Fred R., activity of penicillin in preservation of milk, A173; activity of streptomycin in preservation of milk, A173

Evers, Clifford F., pre-cooked frozen foods, A81; into the freezer—and out, A172

Eyrard, A., the reductose test in quality control, A131

Fabian, F. W., cheese as the cause of epidemics, A147

Faist, Kenneth L., job safety training manual, A23

Farrall, A. W., plans for dairy equipment, A107

Fertiz, John W., coliform studies of pasteurized milk, A182

Fifer, Russell, the butter industry, A54

Filachione, E. W., purification of lactic acid, A70

Fincher, M. G., control of mastitis, A152

Findlay, J. D., ethyl gallate abscorbic acid as antioxidants, A34; use of antioxidants in milk powder, A94; keeping properties of whole milk powder, A99

Fisher, C. H., purification of lactic acid, A70

Fisher, E. H., new insecticides, A158

Foley, E. G., effect of penicillin on mastitis organisms, A36

Forester, T. L., shrinkage of butter, A141

Fouts, E. L., coliform organisms in dairy products, A63

Fraps, G. S., effect of storage on carotene, A95

Frayer, J. M., control of mold growth, A154

Freeman, T. R., coliform organisms in dairy products, A63

Frischknecht, Carl, dried eggs in ice cream, A13; dried eggs in ice cream, A125

Fuel Efficiency Committee, efficient use of fuel, A108

Fulton, C. O., Irish moss extracts as stabilizing agents, A13

Gambrell, G. C., control of dust contamination in agar plates, A25

Geiger, A. A., refrigeration for milk plants, A203

Gelpland, A. J., sweet potato flour in ice cream, A11

Geming, Elizabeth F., effect of temperature on coliform organisms, A62

Geyer, R. P., utilization of galactose, A164

Giberson, E. M., processing of homogenized milk, A131

Gibson, C. A., "winter" flavor of butter, A115

Gibson, D. L., acidity control in creamery butter, A114

Gibson, T., formation of carbon dioxide bacteria, A88

Glazewski, I. G. A., nectar research, A180

Glenn, Dawrence S., freezing foods, A185

Goldblatt, Harry, toxicity of carbon tetrachloride, A156

Golding, N. S., resazurin grade and the standard plate count of milk, A64

Good, E. G., reworking and printing butter, A140

Gordon, W. G., plastics from acylated casein, A55

Gorgenson, S. J., resazurin grade and the standard plate count of milk, A64

Gorini, Costantino, lactic ferments, A205

Gould, I. A. [illegible] ' a. antioxidant in dairy products,' A54

Graham, V. E., overrun and moisture control, A141; variations in the butterfat content of milk, A200; values of cleaners, A205

Grant, E. A., composition of ice cream, A97; ice cream, A123

Grant, H. B., frosted foods, A98

Green, David E., currents in biochemical research, A161

Guilhon, J., milk in the transmission of some protozoa, A119

Gunderson, N. O., control of mastitis, A36

Gundlach, G. P., educating fountain clerks, A12

Gyorgy, Paul, toxicity of carbon tetrachloride, A156

Hadley, C. C., responsibility of milk sanitarians, A203

Hall, J. A., h.t.s.t. pasteurization process, A101

Hall, V. L., postwar milk bottle, A181

Hamilton, F. W., quality of butter, A140; quality of cream, A191

Hamilton, G. R., corn sugar, A128

Hamilton, T. Kline, three-day-a-week delivery, A78

Hammer, B. W., bacteriology of butter, A163

Hankinson, Denzel J., shrinkage defect, A7

Hansen, H. C., vitamin A potency of butter, A2; curing of cheddar cheese, A194

Hanten, A. J., how to stretch sugar, A126

Harmon, E. M., greater butter values shown, A89

Harrison, Thos. B., matting of cottage cheese, A2

Hart, E. B., bacteria in digestive tract, A152; utilization of galactose, A164

Hathaway, I. L., riboflavin content of cheese, A2

Hebner, Anthony, premium milk, A78

Heign, P. C., improved Quebec colony counter, A53

Henderson, J. L., nutritional studies on milk fat, A58; nutritional studies on milk fat, A58; distribution of fatty acids, A164

Henderson, Mattie P., *Salmonella typhirmurium* food infection, A178

Henery, A., milk in the transmission of some protozoa, A119

Hening, J. C., milk for manufacturing, A202

Henry, K. M., biological value of proteins of skim milk, A37; balance of certain milk nutrients cheese, A90; growth-promoting value of fats, A96

Henry, W. C., composition of ice cream, A97

Hesse, Herman C., process equipment design, A170

Hibben, R. C., ice cream sales, A127

Higginbottom, Constance, bacteriological examination of dried milks, A93

Hilditch, T. P., component acids of milk fats, A3; human-milk fat, A3

Hill, T. M., tomorrow's package, A49

Hinshaw, E. B., distribution of fatty acids, A164

Hiscox, Edith R., plate count of spray-dried milk powder, A93; keeping properties of whole milk powder, A99

Hitchcock, John A., saving labor, A6

Hlynka, I., clarifying milk for cheesemaking, A68, the milk clarifier in cheddar cheese manufacture, A174; keeping quality of cheese, A174; making sediment tests, A201

Hodson, A. Z., fluorometric method for thiamin in milk, A26

Hoecker, W. H., bacteriology of butter, A163

Hohl, Leonora A., pretreatment of fruits for freezing, A180

Holm, George E., dried milks, A35

Hood, E. G., clarifying milk for cheesemaking, A68; extraneous matter in cheddar cheese, A146; the milk clarifier in cheddar cheese manufacture, A174; keeping quality of cheese, A174; fat dispersion in agitated milk, A180; making sediment tests, A201

Hoover, R. D., determination of thyroxine in iodinated casein, A134

Houdiniere, A., anemias produced by milk-etiology, A95; techniques applied to the French creamery, A116; a whey cheese, A118; cow's colostrum, A204

Houston, J., balance of certain milk nutrients cheese, A91

Huddleson, Forest I., diagnosis of bovine brucellosis, A152

Huff, A. E., transportation of frozen foods, A45

Huffman, C. F., effect of yeast and pyridoxine on poikilocytosis, A56; bloat in ruminants, A57

Hughey, T. M., refrigerants, A207

Hunter, G. J. E., bacteriophage infection in cheese manufacture, A92

Hunziker, O. F., quality of condensed milk, A150

Hutchings, Barbara L., pre cooked frozen foods, A81

INDERMUHLE, P., extent of inbreeding, A113

Illman, W. I., packaging materials, A159

Irvine, O. R., retention of nutrients, A32; cheddar cheese, A90; the pH of cheese, A147

Irwin, M. R., bactericidal activity of bovine serum, A70

Iverson, C. A., nuts for ice cream, A198

JACK, E. L., nutritional studies on milk fat, A58; nutritional studies on milk fat, A58; distribution of fatty acids, A164

Jackson, H. C., good milk, A40

Jamieson, M. C., swab-slant test for equipment, A31; sanitary control, A206

Jansen, J. F., coliform bacteria in pasteurized milk, A16

Joffe, Max A., vitamin-ed ice cream, A97

Johns, C. K., methylene-blue reduction

test, A60; bacterial content of egg melange, A97
JOHNSON, H. P., detecting streptococcic mastitis, A195
JOHNSON, STEWART, butterfat differentials, A41
JOSEPHSON, D. V., keeping quality of pasteurized milk, A106; keeping quality of milk, A107; new cellulose gum stabilizer, A125; low sugar ice cream mix, A126; freezing and storing condensed skim milk, A175
JOURFRET, R. J., peroxidase reaction, A155

KAHANE, E., the choline substance of milk, A196
KELLEY, C. D., square bottles, A99
KEMMERER, A. R., effect of storage on carotene, A95
KENDALL, T. P., daily advertising survey, A101
KENNEDY, P. G., sweet potato flour in ice cream, A11
KENNEDY, RIDGWAY, JR., lessons of the past four years, A124
KIMBLE, THEODORE H., co-op locker associations, A188
KINGMAN, H. E., blood calcium and inorganic phosphorus, A203
KLEIBER, M., bloat in ruminants, A57
KLOKNER, R. A., refrigeration, A126
KOHLER, GEO. O., determination of thyroxine in iodinated casein, A134
KOLLER, E. FRED, creamery costs, A32
KON, S. K., biological value of proteins of skim milk, A37; balance of certain milk nutrients cheese, A90; growth-promoting value of fats, A96; keeping qualities of whole milk powder, A99
KTOTZER, H. WILEY, truck refrigeration, A21
KULLMAN, T. J., sanitary standards subcommittee, A99
KUPPERMAN, HEBRERT S., neutralization of endogenous gonadotrophic hormone, A72

LA GRUA, J. C., container and packages, A135
LAMPERT, L. M., Babcock test on homogenized milk, A40
LAVAGNE, JACQUES, woman's milk restricted food intake, A123
LAVERS, C. G., packaging materials, A159
LEA, C. H., use of antioxidants in milk powder, A94
LEE, S. W., effect of penicillin on mastitis organisms, A36
LEFFINGWELL, GEORGIA, soap in industry, A87; glycerin—its industrial and commercial applications, A87
LEGGATT, A. G., testing for yeasts and molds, A141
LEHMKUHL, HENRY W., coliform bacteria in pasteurized milk, A15
LEIGHTON, ALAN, homogenized cream in ice cream, A59; technical literature of ice cream, A125
LEMON, J. M., tips on fish freezing, A47
LENNOX, C. E., softening and purifying water, A206
LEPKOVSKY, S., nutritional studies on milk fat, A58; nutritional studies on milk fat, A58
LERNER, E., riboflavin determination, A34
LESSER, MILTON, soap in industry, A87; glycerin—its industrial and commercial applications, A87
LEVY, J. MLLE., the choline substance of milk, A196
LEWIS, DONALD H., rodent control, A167
LEWIS, J. M., vitamin requirements in calves, A57
LEWIS, SAMUEL R., AND ASSOCIATES, cooling towers and evaporative condensors, A79
LIGHTNER, DON, the freezer situation, A129
LONG, LUCY, into the freezer—and out, A172
LORD, J. W., carotene and vitamin A in butter-fat, A121
LUCK, J. MURRAY, annual review of biochemistry, vol. XV, A162
LUKE, H. ALAN, milk delivery in war and peace, A104
LUND, C. E., home freezer development, A186
LYTHGOE, HERMANN C., examination of milk for added water, A62

MACHEREL, PIERRE, fat content of creams, A411

Mallman, W. L., control of micro-organisms in storage rooms, A186
Mann, A. J., butterfat differentials, A41
Mann, T., studies on the metabolism of semen(1), A112; studies on the metabolism of semen(2), A112
Marcellus, F. N., dried whey in chick rations, A196
Marquardt, J. C., cheese improvement program, A3
Marsh, Dorothy B., informative 'labels, A49
Mason, K. M., methylene-blue and direct microscopic tests, A40
Mastermann, Nancy K., moisture losses in stored frozen meats, A82
Masurovsky, B. I., egg solids in ice cream, A9; liquid sugar in ice cream, A10; chocolate ice cream, A38
Mathieu, Solange, woman's milk in restricted food intake, A123
Matthysso, John G., D.D.T., A157
Mattick, A. T. R., keeping properties of whole milk powder, A99
McCallum, D. H., utilization of whey, A117; good pasture, A196
McCracken, Earl C., home freezers, A81
McCrady, M. H., water bacteriology, A111
McCulloch, E. C., disinfection and sterilization, A138; incidence of mastitis, A177
McFarlane, W. D., stability of whole milk powder, A150
McGrory, C. M., plastics from acylated casein, A55
McHenry, E. W., calcium content of cow's milk, A96; ice cream, A123
Meara, M. L., human-milk fat, A3
Merrill, Ariel C., three-day a-week delivery, A79
Merrill, Ezra, marketing efficiencies, A102
Meyer, Roland K., neutralization of endogenous gonadotrophic hormone, A72
Metcalf, Mark, ice cream standards, A127
Metcalfe, B., Irish moss extracts as stabilizing agents, A13
Miollis, Raymond, standards for soft and semi-soft cheese, A55
Molyneux, G. W., milk inspection, A18
Moore, A. V., homogenized milk, A200
Morel, Madeleine, vitamin pp content of cow's milk, A123
Muller-Hoessli, E., calculation of the total solids, A199
Murphy, W. J., infectious hepatitis, A71

Nerenberg, Gloria A., freezing milk in brick form, A19
Newkirk, Stanton M., job safety training manual, A23
Nicholas, John E., milk freezing for commercial use, A84
Nichols, Agnes A., heat resistance of the bacteriophages, A91; bacteriophage and phage-organisms relationships, A92
Noonan, J. J., cabinets—fountains, A129
Nord, F. F., advances in enzymology, vol. 6, A169
Nordisk, Berge S., fertility, A143
Noyes, W. E., thermoduric and thermophilic bacteria, A111; dairy plant sanitation, A135

Olson, T. M., bloat in ruminants, A57
Orians, Betty M., acids of human milk fat, A164
Ostrolenk, Morris, control of dust contamination in agar plates, A25
Outwater, Carson H., transfer of unsatisfactory dairies, A181
Ovson, Leo D., eggs in ice cream, A130
Owens, F. J., fruits-berries, A128

Parfitt, E. H., sanitary standards of the evaporated milk industry, A55
Paul, T. M., component acids of colostrum fat, A204
Payne, Merle G., blood calcium and inorganic phosphorus, A203
Pearce, Jesse A., fluorescence in studying quality, A122; dried milk powder, A148; dried milk powder, A149; dried milk powder, A149; dried milk powder, A149; keeping quality of egg and milk, A150
Penfold, E. E. R., thermometers, A157
Penn, E. G., tomorrow's packages, A43
Perry, C. A., milk sanitation program, A132
Peters, I. I., clarification, A153
Petrie, L. M., infectious hepatitis, A71

Phillips, T. O., how to improve coal utilization, A109; improve coal utilization, A135

Pien, J., determination of fat in creams, A98; graduation of cream butyrometers, A116; dilution of creams, A116; utilization of cheese factory whey, A117; resistance of vitamins to heat, A122; analysis of casein-based, powdered glues, A194; control of pasteurization, A202; analysis of cheese, A193; a new butyrometer, A199; legal definition of cheeses, A193

Plastridge, W. N., mastitis and the plate count, A56

Pond, M. Allen, inspections, A17

Pratt, Mel, new dairy products, A191

Prescott, S. C., water bacteriology, A111

Price, W. V., cheese price, A145; value of whey as a food, A145

Prouty, Charles C., ripening process of American cheddar cheese, A174

Pyenson, Harry, ice cream with reduced sugar, A12

Pyne, G. T., rennet hysteresis, A118

RABUFFO, VINCENT M., analysis of wartime sales, A13; food chains sell ice cream, A81; gallonage hits all time high, A124

Rahn, Otto, microbes of merit, A138; sterilization of microorganisms, A139

Ramsey, R. J., defects in market milk byproducts, A29; defects in by-products, A103; ice cream manufacturing problems, A125; storing ice cream ingredients, A198

Rao, V. R. Bhale, component acids of colostrum fat, A204

Reed, Paul B., refrigerated transport, A185

Reed, P. E., volume in the creamery business, A192

Reed, Paul W., vermin control, A22

Reid, D. F., nutritional studies on milk fat, A58; nutritional studies on milk fat, A58

Reid, F. M., refrigerated trailers, A47

Reid, J. T., effect of yeast and pyridoxine on poikilocytosis, A56

Reineke, E. P., determination of thyroxine in iodinated casein, A134; formation of thyroxine in iodinated casein, A134

Remaley, Robert J., fat problems in dairy products, A74

Renvoise, G., analysis of casein-based, powdered glues, A194

Rey, S., the reductose test in quality control, A131

Rhode, C. S., cost of producing milk, A14

Richards, O. W., improved Quebec colony counter, A53

Riemenschneider, R. W., oxygen absorption of methyl esters, A69

Roberts, S. J., induce heat in the ovariectomized cow, A65

Roberts, W. M., matting of cottage cheese, A2

Robinow, C. F., the bacterial cell, A137

Robinson, Allen D., acidity of fresh milk, A155

Robinton, Elizabeth D., effect of temperature on coliform organisms, A62

Rochaix, A., antagonism of bacteria in contaminated milk, A202

Rosner, L., riboflavin determination, A34

Rottmayer, S. I., cooling towers and evaporative condensers, A79

Rupel, I. W., value of whey as a food, A145

Rushton, J. Henry, process equipment design, A170

SAGER, O. S., phosphatase test for cheese, A55

Salisbury, G. W., livability of bovine spermatozoa, A54

Samson, Herbert S., acidity of fresh milk, A155

Sanders, G. P., phosphatase test for cheese, A55

Sater, Lenore E., home freezers, A81

Sayles, C. I., tomorrow's packages, A183

Schalk, A. F., bloat in ruminants, A57

Schutz, F., adsorption of pepsin and rennin on foam, A118

Scieley, H. W., Jr., mastitis and the plate count, A56

Seifter, Joseph, toxicity of carbon tetrachloride, A156

Semmons, E. M., calcium content of cow's milk, A96

Shelor, Ethyl, ascorbic acid peach pack, A77

Sherman, J. M., coliform bacteria in pasteurized milk, A16

Shuttleworth, P. O., wrapping and packaging materials, A20

Simon, F., antagonism of bacteria in contaminated milk, A202

Simon, W. F., apprenticeship training, A158

Simpson, M., practical h.t.s.t. operation, A130

Slinger, S. J., dried whey in chick rations, A196

Smith, A. K., soybean or vegetable milk, A72

Smith, Edwin, shipment of frozen foods, A184

Smith, J. A., plans for locker plants, A80

Smith, J. A. B., utilization of urea, A6; use of antioxidants in milk powder, A94; keeping qualities of whole milk powder, A99; progress of dairy science, A107

Smith, James T., plant sanitation, A135

Smith, Marjorie, frozen food business, A9

Smith, S. E., applications of coliform tests, A61

Spealman, C. R., effect of temperature on blood flow, A71

Spencer, Leland, economics in milk delivery, A42; milk delivery, A104; milk delivery in war and peace, A104

Sproule, W. H., retention of nutrients in cheese, A32; cooking temperatures for cheddar cheese, A146; extraneous matter in cheddar cheese, A146

Stadtman, Thressa C., sanitation in relation to quality, A183

Stagg, M. V., low temperatures on the highways, A76

Stamberg, Olof E., vitamin A potency of butter, A2

Stanbury, W. M., blood calcium and inorganic phosphorus, A203

Steeves, T. A., reduction of spatial temperature variations, A21

Stirton, A. J., oxygen absorption of methyl esters, A69

Stoltz, R. B., milk containers, A156

Straub, J., phosphatases, A155

Sutheim, George M., introduction to emulsions, A170

Taggart, R. S., insect control, A167

Tanner, F. W., the acid milks, A41

Tapernaux, A., French legislation dealing with concentrated and powdered milks, A93

Tessier, H., sealing vacuum-packed tins, A20

Theophilus, D. R., vitamin A potency of butter, A2

Thibodeau, R., determination of extraneous matter in cheese, A90

Thomas, P. Edwin, insulation and its uses, A20; insulation and its uses, A94

Thomas, S. B., resazurin and methylene blue tests, A151

Thompson, J. B., determination of traces of copper, A175

Thompson, R. J., odorless refrigerants, A23

Thompson, R. W., control of insects, A192

Thompson, S. Y., balance of certain milk nutrients in cheese, A91; keeping properties of whole milk powder, A99

Thomsen, L. C., measuring creamery efficiency, A72; loss of fat in buttermilk, A139

Thomson, F. N., water supplies, A166

Tiedeman, W. D., applications of coliform tests, A61

Tisdall, F. F., ice cream, A123

Tisdall, Frederick, importance of milk in health, A122

Tomarelli, Rudolph, toxicity of carbon tetrachloride, A156

Tracy, P. H., ice cream with reduced sugar, A12; powdered mix in ice cream, A59; market milk industry, A153

Trebler, H. A., dairy cleaners, A159

Tressler, Donald K., merchandising frozen cooked foods, A27; frozen fruit purees, A75; storing frozen foods, A127; into the freezer—and out, A172

Trout, G. M., clarification, A153; homogenized milk, A200

Tucker, C. B., *Salmonella typhimurium* food infection, A178

Tucker, J. Mack, refrigeration abstracts, A66

Turer, J., oxygen absorption of methyl esters, A69

Turner, C. W., determination of thyroxine in iodinated casein, A134; formation of thyroxine in iodinated casein, A134

Turner, G. E., overrun and moisture control, A141; values of cleaners, A205

TUSTIN, E. B., JR., effect of salt on cheddar cheese, A194

VAN BEVER, A. K., phosphatases, A155
VAN DENMARK, N. L., livability of bovine spermatozoa, A54
VAUGHN, REESE H., sanitation in relation to quality, A183

WALLACE, L. R., effect of the diet on foetal development, A95
WALLENFELDT, EVERT, good milk, A40
WATROUS, G. H., JR., a grating type cheese, A26
WEAVERS, HARVEY, sanitation, A65
WEBER, C. W., coliform bacteria in pasteurized milk, A15; h.t.s.t. pasteurizers, A182
WEINREICH, C. F., high-temperature short-time pasteurization, A42
WEISSBERGER, ARNOLD, physical methods of organic chemistry, Vol. 1, A171
WHITE, A. H., "winter" flavor of butter, A115; quality of butter, A140; pH of butter, A191
WHITE, J. C. D., ethyl gallate abscorbic acid as antioxidants, A34
WHITEHEAD, H. R., bacteriophage infection in cheese manufacture, A92
WILCOX, R. H., cost of producing milk, A14
WILEY, W. J., chemistry of butterfat, A148
WILLIGAN, D. A., sanitary control, A206
WILSON, F. L., the acid milks, A41
WILSON, G. S., the resazurin and methylene-blue tests, A100
WILSON, L. T., vitamin requirement in calves, A57
WILSTER, G. H., control of leakiness in butter, A142; manufacturing cheddar cheese, A145
WINSLOW, C. E. A., water bacteriology, A111
WINSOR, KARL, moisture losses in stored frozen meats, A82
WINTER, ASA, control of brucellosis, A176
WINTON, ANDREW L., analysis of foods, A53
WINTON, KATE BARBER, analysis of foods, A53
WOLF, J. Z., heat resistance of the bacteriophages, A91; bacteriophage and phage-organisms relationships, A92
WOOD, EVELYN, diagnosis of bovine brucellosis, A152
WOOD, F. W., standardizing the pH of butter, A140; flavor of butter, A140
WOODROOF, J. G., ascorbic acid peach pack, A77

YOUNG, C. B. F., surface active agents, A171

ZELAYA, J. A., livability of bovine spermatozoa, A54

SUBJECT INDEX OF ABSTRACTS

- ACETYLATED casein, A55
- Acid,
  - ascorbic,
    - as antioxidant, A34
    - in milk products, A178
    - use in fruit processing, A9, A77
  - control in creamery butter, A114
  - fatty, A3, A164
    - of human milk fat, A164
  - lactic, A4, A70
- Acidity of fresh milk, A155
- Adulteration, A166
- Advertising, A101
- Aluminum foil coatings, A107
- Annatto, A93
- Antianemic factors of milk, A6
- Antioxidants, A34, A35, A54, A69, A94, A148
- Apprenticeship training, A158
- Artificial insemination, A54, A112

- BABCOCK test, A40
- Bacteria,
  - *Bacillus licheniformis*, A88
  - *Brucella abortus*, A70, A147
  - Coliform, A14, A15, A16, A61, A62, A63
  - content of egg melange, A97
  - counts, A67
    - direct microscopic, A40
    - plate, A25, A53, A55, A65, A67
  - effect of salt on, A163
  - thermoduric,
    - control of, A111
- Bacteriophage, A91, A92
- Bloat in dairy cattle, A57
- Blood Ca and P of cattle, A203
- Book reviews,
  - Advances in Enzymology, Vol. 6, A169
  - Analysis of Foods, A53
  - Annual Review of Biochemistry, Vol. 15, 1946, A162
  - Bioenergetics and Growth, A1
  - Currents in Biochemical Research, A161
  - Dairy Bacteriology and Public Health, A1
  - Disinfection and Sterilization, A138
  - Enzymes and Their Rôle in Wheat Technology, A162
  - Food Industries Manual, A67
  - Glycerin, Its Industrial and Commercial Applications, A87
  - Industrial Oil and Fat Products, A73
  - Into the Freezer and Out, A172
  - Introduction to Emulsions, A170
  - Microbes of Merit, A138
  - Physical Methods of Organic Chemistry, Vol. 1, A171
  - Piping Handbook, 4th Ed., A73
  - Process Equipment Design, A170
  - Secretion of Milk, 3rd Ed., A111
  - Soap in Industry, A87
  - Surface Active Agents, A171
  - The Bacterial Cell, A137
  - The Refrigeration Data Book, A169
  - Water Bacteriology, A111
- Bottles, square, A99, A102, A133, A181
- Breeding artificial, A54, A111
- Brucellosis, A70, A152, A176
- Business,
  - importance of volume, A192
- Butter,
  - antioxidants for, A54
  - bacteriology of, A163
  - chemistry of, A148
  - color, A31
  - consumption, A28
  - continuous churn, A163
  - control of acid in, A114
  - creamery costs, A32
  - creamery techniques, A116
  - fat loss, A139
  - flavor, A115, A140
  - future of, A116, A142, A152
  - keeping quality, A54
  - molds and yeasts in, A141
  - neutralizing cream for, A31, A140
  - nutritive value, A1, A58, A89, A96
  - oil, A74
  - overrun, A141
  - pH, A191
  - production, A54
  - rancid, A54
  - reworking and printing, A140
  - salt estimation, A143
  - shrinkage, A141
  - vitamin A potency, A2, A25, A89, A121
- Buttermaking,
  - new process, A114, A163

Buttermilk,
  composition, A116
  fat losses in, A139
  use in bread making, A148
Butyrometer,
  graduation of, A116
  new type, A199

CALCIUM,
  content of milk, A96
Calf, dairy,
  vitamin requirements, A57
Carbon tetrachloride,
  toxicity, A156
Carotene,
  effect of storage on, A95
Casein,
  drying, A119
  food from, A56
  from soybeans, A119
  glue, A194
  iodinated, A134
  plastics, A55
Cationic germicides, A166
Cheddar cheese,
  consumption in Canada, A117
  cooking temperature, A146
  cultures, A92
  extraneous matter in, A146
  from pasteurized milk, A145
  nutritive value, A90
  phosphatase test for, A55
  rancidity, A90
Cheese,
  analysis, A193
  annatto coloring, A93
  as cause of epidemics, A147
  bacteriophage in manufacture, A91, A92
  Cheddar,
    cooking temperature, A146
    cultures, A92
    curing temperature, A194
    extraneous matter in, A146
    from pasteurized milk, A145
    nutritive value, A90
    phosphatase test for, A55
    rancidity, A90
    salt, A194
  clarified milk for, A68, A174
  Colby, A178
  consumption, A117
  definition, A193
  extraneous matter in, A90, A174
  grating type, A26
  improvement program, A3
  keeping quality, A174
  nutritive value, A32
  Parmesan, A69
  pasteurized milk for, A69
  plants, A26
  price estimation, A144, A145
  quality control, A3, A90
  riboflavin content, A2
  ripening, A174
  salt estimation in, A143
  standards for, A55
  survival of *Brucella abortus* in, A147
Chocolate ice cream, A129
Choline, A196
Churning, A89, A114
Churns, A163
Clarification of milk, A68
Cleaners, A205
Coal, plant use of, A135
Cold storage, A21
Coliform bacteria, A14, A15, A16
Colostrum, A204
Composite samples, A154
Condensed milk,
  control of quality in, A150
  freezing and storing, A175
  new method for, A34
  sweeteners, A4
Containers,
  fiber, A181
  square bottles, A133, A181
Cooling towers, A79
Copper determination, A175
Costs,
  creamery, A14, A32
  ice cream, A38
Cottage cheese, A2
Cream,
  cooling for churning, A89
  fat determination, A98
  keeping, A19
  neutralization, A31
  quality, A202
  variations in fat content, A154
Creamery efficiency, A72

DAIRY cattle,
  artificial insemination, A54, A112
  breeding, A113, A143

  inbreeding, A113
  photography, A113
Dairy industry,
  future operations of, A153
Dairy plant,
  efficiency, A72
  equipment, A107, A182
  larger size, A26
Dairy products,
  food value of, A2, A6, A25, A32, A37, A58, A89, A90, A96
  new, A191
D.D.T., A157, A158, A167
Delivery, A42, A78, A79, A104
Detergents, A15
Determination of calcium, A7
Determination of salt, A143
Diacetyl, A88
Diet,
  effect on foetal development, A95
Disease,
  dairy cattle,
    bloat, A57
    brucellosis, A70, A152, A176
    mastitis, A35, A36, A56, A119, A152, A177
    poikilocytosis, A56
  food poisoning, A178
  human,
    anemia, A95
    infectious hepatitis, A71
  transmission by milk, A119
Dried milk,
  antioxidants for, A35, A94, A148, A150
  bacterial counts, A93
  biological value of proteins of, A37
  consumer acceptance, A5
  effect of light on, A149
  keeping quality, A99, A149, A150
  legislation, A93
  photolysis of riboflavin in, A149
  preheating, A99
  production, A35
  quality estimation, A93
  solubility, A94
  sorption of CO_2 by, A148

EGGS,
  bacteriological studies, A97
  keeping quality, A150
  use in ice cream, A9, A13, A125, A130
Enzymes, A205
Equipment,
  dairy plant,
    refrigeration, A48
    post war, A107
    sanitation, A31
Ethyl gallate, A34
Evaporated milk,
  standards for, A55
Every-other-day delivery, A78, A79

FAT,
  acids of milk, A3, A164
  antioxidants, A69
  chemistry, A69
  determination, A98
  effect on galactose utilization, A164
  flavor of, A68
  milk,
    effect of cooling on, A180
    food value of, A58
  problems in dairy products, A74
Feeds,
  dried buttermilk, A196
  dried whey, A196
  silage,
    grass, A121
Fluorescence,
  as index of quality, A122
Food,
  flavor, A68
  freezing, A43, A47, A85, A185
  packages, A43, A79, A85, A187, A188
  processing, A183
  storage, A127, A186
Food value of dairy products, A2, A6, A25, A32, A37, A58, A89, A90, A96, A122, A123, A152
Freezers,
  home, A81, A186
  ice cream, A129
Freon refrigeration, A50, A207
Frozen foods, A9, A27, A81, A98
  defrosting of, A66
  fruit purees, A75, A180
  locker plants, A188
  merchandizing, A198
  milk, A19
  packaging, A20, A43, A85, A187, A188
  supervision of, A80, A84
  quality, A183, A184, A187

- transportation, A45, A47, A184, A185
- warehousing, A49

Fruit ice cream, A128
Fruit processing, A180
Fuel,
- efficient use of, A108
- utilization, A109

GALACTOSE, A164
Germicides, A158, A166
Goats,
- milk fat of, A3

Grass silage,
- chemistry of, A121

Grating type cheese, A26

HOMOGENIZED milk,
- Babcock test for, A40
- processing, A131
- selling, A78

Human milk fat, A3
Hydrogen ion concentration,
- dairy applications, A175

ICE cream,
- cabinets, A129
- chocolate, A38
- composition, A97
- costs, A38
- dipping, A198
- eggs in, A9, A13, A125, A130
- first manufacture of, A9
- food value of, A123
- freezers, A129
- fruit, A75
- homogenized cream in, A59
- ingredient storage, A198
- low sugar, A12
- manufacture, A71, A125
- nuts in, A198
- penicillin in, A126
- plant construction, A126
- plant operation, A12
- powdered mix, A59
- production, A8
- quality control, A9
- review of literature on, A125
- sales, A13, A39, A124, A127
- selling, A12, A38, A39, A179
- shrinkage defect, A7
- stabilizers, A7, A13, A125
- standards, A127
- sugar for, A8, A10, A126, A128
- sweet potato flour in, A11
- use of eggs in, A9, A13
- vitamined, A75, A97

Infectious hepatitis,
- milk-borne, A71

Insect control, A157, A158, A167, A192
Insemination, artificial, A54, A112
Insulation, A20, A46
Irish moss, A13

JOB training, A23

LABOR,
- saving, A6
- use on farms, A6

Laboratory control, A14
Lactic acid,
- manufactured from whey, A4
- purification, A70

Lactose determination, A4
Lecithin,
- in chocolate ice cream, A38

Legislation, A93
Leucocyte count of milk, A36
Locker plants, A80

MARKETING, A102
Mastitis,
- control, A35, A36, A152
- detection, A36, A195
- incidence, A177
- penicillin treatment of, A36, A119
- treatment, A36, A119

Merchandising,
- frozen foods, A27
- ice cream, A179

Methylene blue test, A40, A60, A100, A151
Milk,
- acid, A41
- antianemic properties of, A6
- bottles, A133, A181
- butterfat content, A200
- calcium content, A96
- clarification, A153
- condensed, A34, A175

consumption, A28
containers, A156, A181
defects, A29, A103
delivery, A42, A78, A79
distribution, A28, A104, A132
dried, A5, A34, A35, A37
evaporated, A55
fat acids, A3, A164
fat dispersion in, A180
freezing, A19
grading, A60
homogenized, A40, A78, A131
human, A123
inspection, A17, A18, A181
marketing, A102
pasteurization, A27, A42, A101, A105, A130, A182
pasteurized,
  coliform bacteria in, A14, A15, A16, A61, A182
  keeping quality of, A106, A107
  laboratory control, A14
preservation, A173
production costs, A14
production trends, A17, A28
purchasing plans, A41
quality control, A14, A15, A16, A40, A41, A100, A131, A132, A165
rancidity, A200
sales, A131
sanitary standards, A99
thiamin determination, A26
total solids content, A199
vitamins in, A122, A123, A178
Milk houses, A18

NUT ice cream, A198

PACKAGES,
  food, A43, A49, A79, A183, A187, A188
  paper, A135, A181
  labels for, A49
  materials, A159
  rubber hydrochloride film, A85
Parmesan cheese, A69
Pasteurization,
  equipment, A27, A182
  methods, A42, A101, A105, A130
Pasteurized milk,
  coliform bacteria in, A14, A15, A16, A61
  for cheddar cheese, A145
  keeping quality, A105, A107
  laboratory control, A14
Pastures, A196
Penicillin,
  effect on spores in milk, A173
  in ice cream A126
  in mastitis treatment, A36, A119
Pepsin,
  absorption on foam, A118
Peroxidase reaction, A155
Phosphatase test,
  for cheddar cheese, A55
  for pasteurization control, A155
Phosphorus,
  retention in cheese making, A32
Physiology,
  of circulation, A71
  of lactation, A107
  of reproduction, A65, A72, A107
Plant equipment,
  post war, A12
  pasteurization, A182
  refrigeration, A21
  sanitation, A135
Plastics,
  from casein, A55
Plate counts, A25, A56, A67
Poikilocytosis, A56
Production,
  costs, A14
Protein,
  biological value, A37
Purchasing plans, A41

QUALITY,
  butter, A31, A140, A141, A142
  cheese, A3, A174
  condensed milk, A150
  cream, A191
  dried milk, A99, A148, A149
  ice cream, A9
  milk, A65, A99, A100, A103, A133, A165
    adulteration, A62, A166
    bacteria counts, A40, A64
    coliform count, A14, A15, A16, A61, A62, A63
    basic essentials, A40
    grading, A60, A64
    inspection, A17, A62, A154, A181
  processed foods, A183, A184
Quebec Colony Counter, A53

REFRIGERANTS, A23, A50, A207
Refrigeration, A21, A48, A50, A63, A66, A76, A126, A185
Rennin,
  absorption on foam, A118
  purification, A33
  hysteresis, A118
Reproduction,
  physiology of, A65, A72, A107
Resazurin test, A100
  effect of udder cells on, A151
  use in milk grading, A64
Reviews, book,
  Advances in Enzymology, Vol. 6, A169
  Analysis of Foods, A53
  Annual Review of Biochemistry, Vol. XV, 1946, A162
  Bioenergetics and Growth, A1
  Currents in Biochemical Research, A161
  Dairy Bacteriology and Public Health, A1
  Disinfection and Sterilization, A138
  Enzymes and Their Rôle in Wheat Technology, A162
  Food Industries Manual, A67
  Glycerin, Its Industrial and Commercial Applications, A87
  Industrial Oil and Fat Products, A73
  Into the Freezer and Out, A172
  Introduction to Emulsions, A170
  Microbes of Merit, A138
  Physical Methods of Organic Chemistry, Vol. 1, A171
  Piping Handbook, 4th Ed., A73
  Process Equipment Design, A170
  Secretion of Milk, 3rd Ed., A111
  Soap in Industry, A87
  Surface Active Agents, A171
  The Bacterial Cell, A137
  The Refrigeration Data Book, A169
  Water Bacteriology, A111
Riboflavin,
  content of cheese, A2
  determination, A34
  retention in cheese, A32
Rodent control, A22, A167
Ruminant,
  utilization of non protein nitrogen, A6

SALES,
  ice cream, A13, A38, A39, A124
  milk and cream, A78
  opportunities, A134
Salesmanship,
  training, A12
Sanitary standards, A99
Sediment test, A201
Semen,
  metabolism, A112
  preservation, A54
Shrinkage defect, A7
Silage,
  grass, A121
Soft curd milk, A103
Soybean milk, A72
Spermatozoa, A54, A112
Stabilizers, A7, A13
Sterilizing operations, A139
Storage,
  effect on dry milk, A37
Streptomycin, A173
Sucrose determination, A4
Sugar, A10, A12, A128, A197
Sweet potato flour in ice cream, A11

TECHNIQUES,
  bacteriological, A31
  copper determination, A175
  riboflavin determination, A34
Thermoduric bacteria, A111
Thermometers, A157
Thyroprotein, A134
Transportation,
  refrigerated, A76, A184, A185
Trucks,
  refrigeration, A21, A76

UDDER cells, A151
Urea,
  utilization by ruminants, A6

VACUUM packing, A20
Vermin control, A22, A167, A192
Vitamins,
  A,
    value of butter, A2, A25, A89

- Ascorbic acid,
  - in milk products, A178
  - use in fruit processing, A9, A77
- Carotene,
  - effect of storage on, A95
- Choline, A196
- D,
  - milk, A103
- in ice cream, A75, A97
- Nicotinic acid,
  - content of dairy products, A123
- pyridorin, A56
- requirement of calves, A57
- resistance to heat, A122
- Riboflavin,
  - content of cheese, A2, A32
  - determination, A34

WATER supplies, A166, A206

- Whey,
  - cheese from, A118
  - food value of, A117, A145
  - lactic acid from, A4
  - utilization, A117

YAMI yogurt, A19

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